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**Growth and Development in the Ediacaran Macrobiota**

# Growth and development in the Ediacaran Macrobiota

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# Thesis Abstract:

The evolutionary emergence and subsequent radiation of the animals remains one of the most significant events in Earth history. The fossil record has historically been considered to give up uncontroversial evidence for the existence of animals only at the Precambrian – Cambrian boundary, ~539 million years ago. However, molecular clocks consistently indicate a much more ancient origin for this group, deep in the Tonian or Cryogenian Periods, 700–800 million years ago. This mismatch is a problem of unusual significance because the animal fossil record is often used as a proxy with which to test hypotheses on the evolutionary process itself. Until we understand these early stages in animal evolution, such hypotheses will remain unvalidated. Some of the best Precambrian candidate animal fossils are members of the Ediacaran Macrobiota, a soft-bodied assemblage of organisms that appear in the fossil record ~571 million years ago, but have a controversial reseach history.

This thesis turns to the study of growth and development as an underexplored avenue with which to shed new light on the affinities of these fossils. I examine populations of taxa from multiple localities, and use the study of comparative morphology, including both X-ray microtomography and synchrotron radiation X-ray microtomography in order to create new models of anatomy and, using these data, quantify morphogenetic patterns. These new data allow phylogenetic analyses to be undertaken in order to resolve the positions of my studied taxa. I find support for a crown-group metazoan affinity for members of both the rangeomorphs and the arboreomorphs. These data provide further support to the hypothesis that the 'Cambrian Explosion' of metazoan taxa has a protracted Neoproterozoic fuse. Perhaps the Cambrian radiation of animal groups represents one of a series of metazoan radiations that began in the late Neoproterozoic.

# Author's Declaration:

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

F. S. Dunn

11-07-2019

# Statement of collaboration:

Chapters 1, 2, 3, 4 and 5 are research collaborations led by the author, with personal contributions outlined at the start of each chapter. Chapter 6 is entirely the work of the author.

# Thesis Acknowledgements:

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*And on the pedestal these words appear:*
*'My name is Ozymandias, King of kings;*
*Look on my works, ye Mighty, and despair!'*
*Nothing beside remains. Round the decay*
*Of that colossal wreck, boundless and bare*
*The lone and level sands stretch far away.*

- Percey Shelley, Ozymandias

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# List of abbreviations:

**CGSM**: Central Siberian Geological Museum, Novosibirsk, Russia

**BGS**: British Geological Survey, Keyworth, UK

**GSM:** Geological Survey Museum, UK

**SAM:** South Australia Museum, Australia

**CAMSM**: Sedgwick Museum, University of Cambridge, UK

**LEIUG**: Department of Geology, University of Leicester, UK

**PIN**: Palaeontological Insititute, Russian Academy of Sciences, Moscow, Russia

**OUMNH**: Oxford Museum of Natural History, Oxford, UK

# Chapter 1:

# General introduction

**Author contributions:** Parts of this chapter are published in *Palaeontology Online, Nature Ecology and Evolution* and *Biological Reviews.*

**Dunn, F. S.** and Liu, A. G. Fossil Focus: The Ediacaran Biota. *Palaeontology Online.* ***7***(1), 1–15.

**Dunn, F. S.** and Liu, A. G. 2019. Viewing the Ediacaran Macrobiota as a failed experiment is unhelpful. *Nature Ecology and Evolution.*

**Dunn, F. S.,** Liu, A. G. and Donoghue, P. C. J. 2018. Ediacaran Developmental Biology. *Biological Reviews.* ***93***(2), 914–932.

Fossil Focus: The Ediacaran Biota; F.S.D and A.G.L designed the study, and wrote the manuscript. Viewing the Ediacaran Macrobiota as a failed experiment is unhelpful; F.S.D designed the study and produced the first draft of the manuscript. Ediacaran Developmental Biology: F.S.D, A.G.L and P.C.J.D desgined the study and F.S.D produced the first draft of the manuscript. All authors provided feedback on the manuscripts. F.S.D is the lead author of all three papers. F.S.D contributed ~90% of the work presented in this chapter.

## 1.1 The Origin of Animals

The Neoproterozoic Era – the vast stretch of time from 1000 to 541 million years ago [Ma] – arguably encompasses one of the greatest periods of geological and biological change in the history of the Earth System (Fig 1.1). It records the point of departure from the microbially-dominated world that characterised much of Earth history to the establishment of Phanerozoic-style ecosystems that have shaped the last 541 million years.

Neoproterozoic time is divided into three Systems – the Tonian (1000–720 Ma), the Cryogenian (720–635 Ma) and the Ediacaran (635–541 Ma). The Tonian sees the beginning of the breakup of the supercontinent Rodinia (Fig. 1.1B), after which carbon isotope values fluctuate increasingly until they crash at the base of the Cryogenian Period, marking the descent into Icehouse conditions (e.g. Shields 2017). The Cryogenian Period is colloquially termed 'Snowball Earth', and is marked by two great Ice Ages – the Sturtian (717–660 Ma, Rooney *et al.* 2015), and the Marinoan (>639–635 Ma, Prave *et al.* 2016). The release from Icehouse conditions at the terminal Marinoan marks the beginning of the Ediacaran Period, which experienced one final pulse of glaciation (this is not considered to have been a global event, but is known from eight palaeocontinents, Hoffman and Li 2009; McGee *et al.* 2013), termed the Gaskiers glaciation at ~580 Ma (Pu *et al.* 2016). Phosphorus, a biolimiting element, was present in seawater at up to 400 ppm during the Ediacaran Period, and so favouring increased primary productivity, before declining to ~200 ppm at the Precambrian-Cambrian boundary (Shimura *et al.* 2014), and there is recorded the largest negative carbon isotope excursion on record – the Shuram/Wonoka anomaly (the timing and cause of which remains uncertain, e.g. Grotzinger, Fike and Fischer 2011).

It is during this period of great geological and geochemical upheaval that molecular clocks, which estimate divergence times based on the differences in molecular genetic sequences, suggest the animals originated (~700–800 Ma, e.g. dos Reis *et al.*, 2015). However, unequivocal fossil evidence for animal communities with representatives of extant animal phyla is not found until closer to ~541 Ma (e.g. Erwin *et al.*, 2011; Cunningham *et al.*, 2017). This mismatch between different records of life is difficult because fossils and divergence time estimation reciprocally rely on each other; more precise divergence time estimates for

animals require a greater number of fossil calibrations from early diverging groups (Dos Reis *et al.* 2015; Pisani and Liu 2015), the very fossils that remain enigmatic, but we also know that the first fossil appearance of a group does not reflect their origin. Members of the Ediacaran Macrobiota, a probably polyphyletic assemblage of soft-bodied organisms that dominate fossil assemblages for the 30 million years before the Cambrian Period, are often invoked as antecedents to these extant clades, with the current broad consensus being that many of these organisms are allied to early-branching lineages of Metazoa or Eumetazoa (e.g. Xiao and Laflamme, 2009; Budd and Jensen, 2017). Surprisingly, in many cases this consensus does not rest on material evidence of metazoan affinity, but on an implicit assumption that the organisms are total-group metazoans (e.g. Budd and Jensen 2017). Ediacaran taxa are invoked increasingly in debates on the origin and evolution of metazoan developmental novelties, including the specification of primary body axes, the making and breaking of axial symmetries, and the appearance of metamerism and/or segmentation (e.g. Malakhov, 2004), but an animal affinity remains to be demonstrated.

Determining the correct phylogenetic position of Ediacaran macrofossil taxa, or even being able to provide convincing positive evidence for an unquestionably metazoan placement in some cases, is a challenge with significant consequences for understanding metazoan development and morphogenesis. However, our ability to close the gap in our understanding of early animal evolution between the rocks and the clocks is hampered by several factors:

1) The fossil record is incomplete and non-uniform, recording only snapshots of ecosystems in certain environments at certain times, and this impacts our understanding of evolutionary patterns (e.g. Benson *et al.* 2010; Flannery-Sutherland *et al.* 2019). This problem is accentuated at the Precambrian-Cambrian boundary, which is marked by a “Great Unconformity”; Cambrian to Ordovician rocks lie unconformably atop Neoproterozoic sediments, the result of continental denudation followed by marine transgression (e.g. Keller *et al.* 2019). This means that the rocks we would expect to record the early radiation of the animals are missing. However, these mechanisms have also been invoked as a trigger for the ‘Cambrian Explosion’ (e.g. Peter and Gaines 2012).

2) In recent years, the study of molecular phylogenetics has helped to resolve a number of outstanding questions (e.g. resolving the probable monophyly of the poriferans; reviewed in Dunn *et al.* 2014), but ambiguity regarding the inter-relationships of many animal clades remains. These include the positions of Porifera and Ctenophora (e.g. Simion *et al.* 2017; Whelan *et al*. 2017; Fueda *et al.* 2017), the position of Placozoa and the reality of Planulozoa (e.g. Wallberg *et al.* 2004; Laumer *et al.* 2018) and inter-relationships of Superphyla like Spiralia (Dunn *et al.* 2014; Laumer *et al.* 2015; Marletaz *et al.* 2019). This is significant because different phylogenetic trees will polarise characters and thus patterns of character acquisition in different ways, changing our perception of how early animals may have looked and behaved. Futhermore, given that stem-group members of a clade are expected to possess only a subset of characters used to diagnose that group (and in many cases diagnostic characters remain controversial), resolving stem-group members of, particularly, higher-order groups remains challenging (see discussion in Cunningham *et al.* 2017).

3) Many Neoproterozoic candidate animal fossils possess unusual anatomies, often not easily reconciled with living taxa. This has resulted in a slew of different phylogenetic interpretations for many of these fossils, spanning much of the eukaryote tree (for example, concerning frondose Ediacaran macrofossils: Pflug 1972; Pflug 1973; Glaessner 1984; Buss and Seilacher 1994; Retallack 1994; Seilacher, Grazhdankin and Legouta 2003; Peterson 2003; Budd and Jensen 2017). Furthermore, many are preserved exclusively as cast and moulds, meaning that only what is interpreted to be the exterior of the organism is preserved. There are reported cases of putative internal anatomy, but these are rare (though see Narbonne *et al.* 2013; Sharp *et al.* 2017).

By more extensive study of Neoproterozoic candidate animal fossils we may hope to better understand their anatomies and perhaps refine their phylogenetic placement. This, in turn, may help in resolving outstanding questions in animal phylogenetics, and to rationalise between the different records of life – the rocks and the clocks.

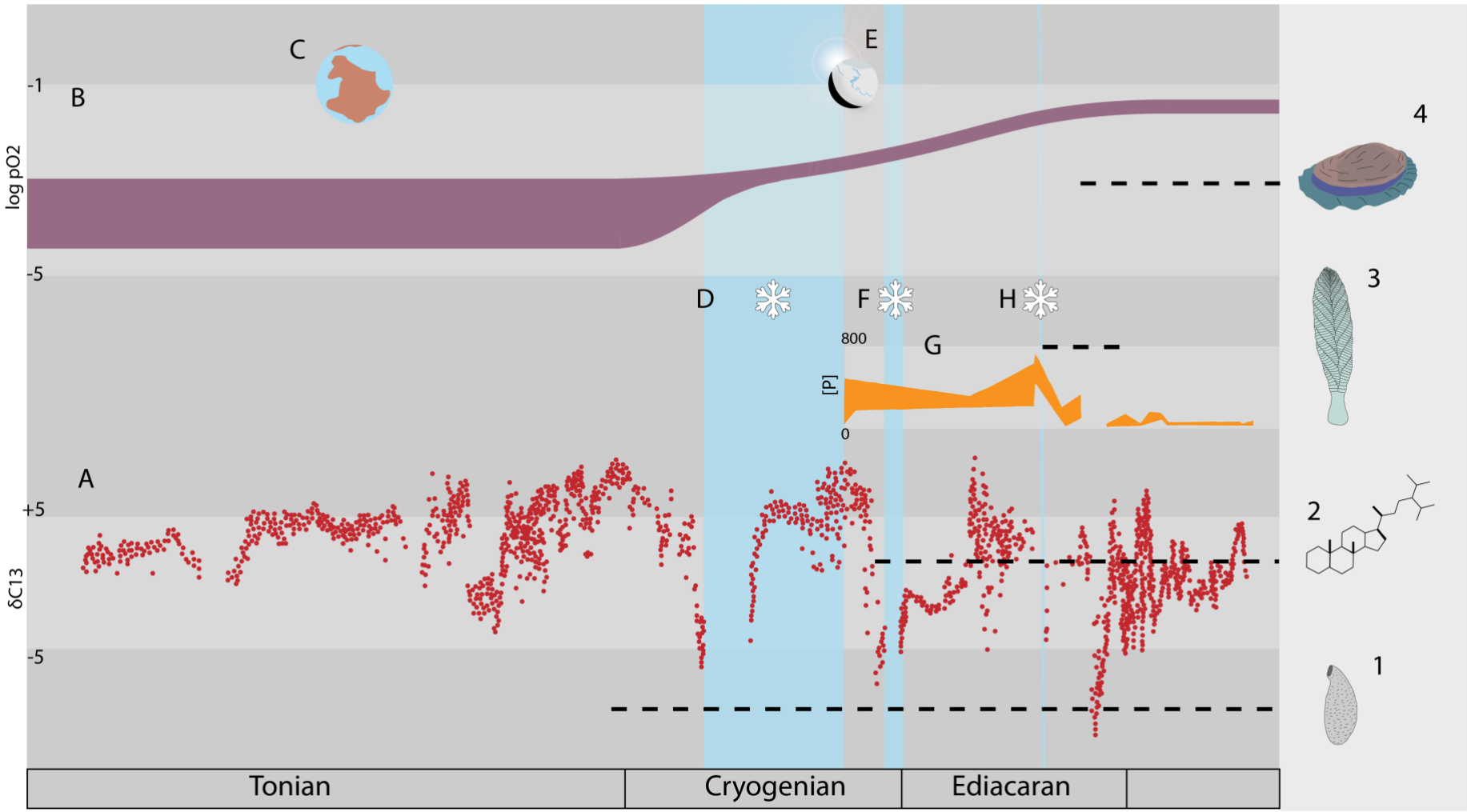


**Figure 1.1:** Major geological, geochemical and biological transitions to occur during the Neoproterozoic. **A)** Carbon (δ13C) values across the Neoproterozoic, data from Butterfield 2015 **B)** Projected changes in oxygen concentration (log *p*O2) during the Neoproterozoic, data from Lyons *et al.* 2014. **C)** The beginning of the breakup of Rodinia, **D)** The Sturtian Glaciation, **E)** The Acraman Event (meteorite impact), **F)** The Marinoan Glaciation, **G)** Seawater phosphorus concentration, data from Shimura *et al.* 2014 **H)** Gaskiers Glaciation. **1)** Fossil evidence for amoebozoan grade organisms **2)** Presence of 24-isopropylcholestane, a potential demosponge biomarker (although this has recently been disputed; Nettersheim *et al.* 2019), **3)** Range of the classical Ediacaran Macrobiota, **4)** Record of probable bilaterian animals. There remains ongoing debate surrounding the correlation between geochemical events, geological events and biological events, as well as the timing of many of these.

## 1.2 The Ediacaran Period

### 1.2.1 Fossils of the Ediacaran Period

Fossils of the Ediacaran Period have a long research history. Between the 1850s and 1870s, interesting impressions were found in England and Newfoundland, Canada, on bedding planes that pre-dated known Cambrian fossils (Salter 1856; Salter 1857; Billings 1872). It was not clear to their discoverers whether these often circular structures were truly the remains of once-living organisms, or were the result of unrelated sedimentary processes. The widely held opinion at the time was that there was no life before the Cambrian; older rocks were called Azoic, meaning 'without life', and the known fossil record strongly suggested that life radiated from nowhere into an astounding diversity of forms during the Early Cambrian, around 520 million years ago (reviewed in Gould 1989). This paradigm discouraged the early discoverers from seriously considering that their impressions could have been biological in origin, but contradicted the long ancestry of complex life predicted by Darwin's theory of evolution by natural selection (Darwin 1859). In the 1930s and 1940s, a range of more complex impressions were found in Russia, Namibia and South Australia (Gürich 1933; Sprigg 1947; Keller *et al.* 1974). Although it was clear that these fossils recorded biological remains, their actual age could not be determined. The Russian material lay in sediments that had previously been mapped as dating from the Devonian Period (419–359 Ma; Murchison 1849), whereas in Australia, the possibility that the fossils were lowest Cambrian (Sprigg 1947) could not be ruled out.

The situation changed in 1957 with the discovery and publication of a frondose fossil named *Charnia masoni* (Ford 1957), which was found on a bedding plane in England that was demonstrably older than the Cambrian. The similarities between *Charnia* and some Australian and Russian fossils (particularly other frondose forms) enabled Martin Glaessner (Glaessner 1961) to recognize that globally distributed communities of soft-bodied organisms had existed, and thrived, well before the base of the Phanerozoic, vindicating Darwin's gradualistic ideas of evolution by natural selection. The organisms were collectively termed the Ediacara biota, after the Ediacara Hills in South Australia, and have since been found globally. They have been joined by a variety of other late Ediacaran fossils that are not found

at the original Ediacara site, and do not represent the remains of originally soft-bodied organisms (e.g. Penny *et al.* 2014). As a result, 'Ediacara biota' has become a less clear-cut term, and in this thesis I use 'Ediacaran Macrobiota' to refer to all macroscopic soft-bodied organisms of late Ediacaran age.

While at the time of writing the Ediacaran Period lacks formal subdivision, it is considered that the Period should be split into two Series, the lower characterised by acanthomorphic acritarchs, and the upper characterised by Ediacara-type macrofossils (Xiao *et al.* 2016). This would place the boundary between the Series between ~580 – 571 Ma. This thesis focuses on the fossils of the Upper Series.

### 1.2.2 The Ediacaran Macrobiota

The Ediacaran Macrobiota are typically split into three 'assemblages': the Avalon, White Sea and Nama (e.g. Waggoner 2003; Boag *et al.* 2016). The assemblage concept is sometimes used to impose an evolutionary trajectory on the record of Ediacaran macrofossils: Avalon-type to White Sea-type to Nama-type biotas (e.g. Droser and Gehling 2015; Boag *et al.* 2019). Statistical evidence appears to support the biological reality of these assemblages (e.g. Boag *et al.* 2016). However, recent work has shown that these biotas are all preserved in distinct depositional environments which lack a continuous rock record. It is, therefore, becoming more difficult to argue that the three assemblages represent an evolutionary succession.

The oldest of these assemblages is the Avalon. These fossils can be preserved in dense assemblages, with ~40 per square meter on the E surface of the Mistaken Point Formation, Newfoundland (~565 Ma) (Clapham, Narbonne and Gehling 2003), but there is little compelling evidence to suggest Avalonian organisms were present in rocks of pre-Gaskiers age. These communities are known primarily from the east coast of Newfoundland, Canada, and Leicestershire, UK, and record deep-water slope and basinal facies (e.g. Carney 1999, Wood *et al.* 2003; Narbonne 2005; Ichaso *et al.* 2007; Mason *et al.* 2013), with organisms typically preserved under ash beds or volcaniclastic sediments (Conception-style; Narbonne 2005; Liu 2016), or within or on the soles of turbidites/contourites (e.g. Brasier *et al.* 2013).

Two morphogroups dominate the Avalon assemblages: the rangeomorphs (Fig. 1.2A-C), and the arboreomorphs (Erwin *et al.* 2011, Fig. 1.2D). Both were sessile, frondose groups, and both are amongst the most enigmatic of the Ediacaran Macrobiota (e.g. Xiao and Laflamme 2009). The rangeomorphs, defined by the presence of at least three orders of self-similar branching architecture, are the most speciose Ediacaran morphogroup with 13-15 genera (at the time of writing), and the stratigraphically longest-ranging, being present until almost the close of the Ediacaran Period (e.g. Darroch *et al.* 2018). The arboreomorphs, known from 5 genera, appear to show significant disparity in heavily-weighted taxonomic characters (e.g. branching architecture; Deccechi *et al.* 2017; Hoyal Cuthill and Han 2018), and so the current definition of the group may require reappraisal (Laflamme, Gehling and Droser 2018).

Rangeomorphs and arboreomorphs numerically dominate communities, but the Avalon assemblage also consists of many other taxa; *Parviscopa bonavistensis* is a multi-branched fossil with non-rangeomorph branching elements present at the tips (Hoffman, O'brien and King. 2008); *Hadryniscala avalonica* is a bizarre ladder-like fossil that could reach over a metre in length (Hoffman *et al.* 2008); *Thectardis avalonensis* is a triangular shaped fossil, common on some of the oldest preserved bedding surfaces (Clapham *et al.* 2004), and previously interpreted as a sponge (Sperling, Peterson and Laflamme 2011; though see Antcliffe 2014); *Haootia quadriformis* (Fig 1.2J) is a goblet shaped organism interpreted as a total group cnidarian (Liu *et al.* 2014). There are also rare examples of surface-locomotory traces in the Avalon assemblage, which provide some support for motile metazoans (Liu, McIlroy and Brasier 2010), although the trace-makers remain unknown.

The Avalonian communities host a number of organisms with different bodyplans, and different hypothesised affinities (e.g. *H. quadriformis*; a crown-group eumetazoan [Liu *et al.* 2014, or *Palaeopaschichnus sp.*; a protozoan [Antcliffe, Gooday and Brasier 2011]). They record the first evidence of probable metazoan locomotion, as well as one of the first probable adaptive radiations in the fossil record, with the rapid diversification of Rangeomorpha (e.g. Shen *et al.* 2008; Hoyal Cuthill and Conway Morris 2014). The Avalon biotic assemblage records a crucial timeslice in the transition from the microbially-dominated Proterozoic Eon, to the dynamic ecosystems of the Phanerozoic Eon (e.g. Butterfield 2015).

The White Sea assemblage, named after the Russian sections, but also (famously) known from the Flinders Ranges of Australia, preserves communities in pro-deltaic shelf settings (e.g. Gehling and Droser 2013). A number of the taxa characteristic of this assemblage can be associated with trace fossils that are commonly inferred to represent feeding or lomomotory traces (though see McIlroy, Brasier and Lang 2009). Some of these taxa, including *Kimberella quadrata* (Fig. 1.2G) and *Dickinsonia* sp. (Fig. 1.2H) are widely considered to represent the remains of ancient animals (Fedonkin and Waggoner 1997; Fedonkin 2007; Ivantsov 2010; Hoekzema *et al.* 2017; Evans *et al.* 2017; Bobrovskiy *et al.* 2018), but where exactly they sit in metazoan phylogeny is the subject of debate (e.g. Fedonkin 2007; Ivantsov 2009; Arendt *et al.* 2015; Hoekzema *et al.* 2017; Evans, Droser and Gehling 2017). Some trace fossils from this time, for example *Helminthoidichnites* (Fig. 1.2E), are sometimes inferred to have been made by bilaterian-grade organisms (Droser and Gehling 2015). *Helminthoidichnites* is the most common ichnofossil in the White Sea assemblage but may also be found in rocks of Cambrian age (e.g. Jago and Gatehouse 2007), suggesting that the presumed metazoan trace-maker may have been long-ranging.

Frondose forms dominant in the Avalon assemblage are present but are typically rare components of these ecosystems. There are two notable exceptions to this. The generalist rangeomorph *Charnia masoni* (Fig. 1.2A) has the ability to exist in single-taxon communities (on storm-influenced middle shorefaces; Grazhdankin 2004), or where other more typical White Sea taxa are less common, alongside other rangeomorph taxa (e.g. flat-laminated to linguoid-rippled sandstone facies; Gehling and Droser 2013, Droser *et al.* 2017). The archetypal arboreomorph *Arborea arborea* (Fig. 1.2D) – previously described only from South Australia – could live alongside classic White Sea taxa (e.g. *Spriggina*, see Fig. 1.2F) in greater densities than rangeomorphs (e.g. wave-base Sands; Gehling and Droser 2013).

The radialomorphs (encompassing triradialomorphs, tetraradialomophs and pentaradialomorphs; Erwin *et al.* 2011) - appear in these fossil assemblages, and are characterised by various excursions into forms of radial symmetry, e.g. the triradial *Tribrachidium heraldicum* (Glaessner and Wade 1966), the pentaradial *Arkarua adami* (Gehling 1987), or the octo-radial *Eoandromeda* (e.g. Zhu *et al.* 2008). Hypotheses of affinity for some radialomorphs exist, and are typically metazoan (e.g. a ctenophoran affinity for

*Eoandromeda octobranchiata* [Tang *et al.* 2009], or an echinoderm affinity for *A. adami* [Gehling 1987]), and these have achieved various levels of support. These forms could represent important parts of White Sea communities, and in some cases (e.g. the triradialomorphs) record symmetry states that are not known from Phanerozoic animals (e.g. Clites *et al.* 2018).

The White Sea biotic assemblage records widespread evidence for animal life, with compelling evidence to suggest that many of these organisms were either fully motile (Ivantsov and Malakovskaya 2002; Fedonkin 2007; Ivantsov 2009), or were at least able to preferentially orient themselves (Paterson *et al.* 2017; Darroch *et al.* 2017; Coutts *et al.* 2018). The interpretation of *Kimberella quadrata* as a bilaterian organism, and a probable protostome (Benton *et al.* 2015; Fedonkin and Waggoner 1997; Ivantsov 2009) remains the oldest evidence for triploblastic animals, supported by the emergence of bilaterian-grade trace fossils described above. These do not represent the only new body plans to emerge in this biotic assemblage with other conspicuous additions including the radialomorphs and tubular body fossils, indicating diversity in the shallows by this time.

The Nama assemblage records latest Ediacaran deposits (~549–541 Ma), and evidences a declining number of soft-bodied groups. The Erniettomorpha (Erwin *et al.* 2011; Fig. 1.2K) are the dominant soft-bodied morphogroup (although they are recorded in the White Sea assemblage), and are characterised by an anatomy constructed of tubular structures that may have been filled with sand during life (e.g. Grazhdankin and Seilacher 2002; Ivantsov *et al.* 2016). The Nama assemblage is sometimes referred to as depauperate (e.g. Darroch *et al.* 2015) because it records a decline in the number of soft-bodied taxa; dickinsoniomorphs, kimberellomorphs, bilaterialomorphs, radialomorphs and arboreomorphs (after Erwin *et al.* 2011) all present in the White Sea assemblage are absent in the Nama assemblage. Rangeomorphs are also scarce, being largely represented by single taxon; *Rangea schneiderhoehni* (e.g. Vickers-Rich *et al.* 2013).

Tubular fossils that begin to appear in the White Sea assemblage radiate (summarised in Boag, Darroch and Laflamme 2016; Darroch *et al.* 2018), with the appearance of forms including *Corumbella werneri* (Babcock *et al.* 2005, Fig. 1.2I) but perhaps the most striking

new arrivals were biomineralizing organisms with calcium carbonate skeletons, such as *Cloudina* or *Namacalathus hermanastes* (e.g. Wood 2011; Penny *et al.* 2014). Some authors consider that *Cloudina* formed some of the first reef structures (e.g. Penny *et al.* 2014), though others suggest that the dense assemblages of *Cloudina* represent transported deposits (Mehra and Maloof 2018).

The trace fossil record also witnesses a change in the Nama assemblage, with an increase in burrowing depth and sediment bioturbation from the White Sea assemblage (Mángano and Buatois 2014). The Nama assemblage also hosts the first record of meiofaunal bilaterian burrows (Parry *et al.* 2017) and shows that both macro and meiofaunal bilaterians had occupied infaunal niches by this time.

Some workers attribute the decline of soft-bodied Ediacaran fossils in these deposits to the rise of tubular and biomineralising taxa in a 'biotic replacement' model. This proposes that the emergence of bilaterian animals, with the ability to engineer their environments, made these same environments largely unfavourable to classical Ediacaran organisms (Laflamme *et al.* 2013; Darroch *et al.* 2015). However, others suggest that there is little evidence for the common spatial overlap in these late Ediacaran groups (e.g. Wood *et al.* 2019), which would be required for biotic replacement to have taken place. An alternative catastrophe-based model attempts to link global geochemical events and the decline of the Ediacaran Macrobiota (e.g. Darroch *et al.* 2018), but the exact timing of these events (e.g. the Shuram excursion or the Basal Cambrian Isotope Excursion) remains contentious (e.g. Darroch *et al.* 2018; Wood *et al.* 2019). Furthermore, many of the tubular and biomineralising taxa that are indicative of the terminal-Ediacaran Period – and are implicated in the biotic replacement model – do not appear to continue into the Cambrian Period, and this has led to hypotheses of a two-phase or 'pulsed catastrophe' event (e.g. Darroch *et al.* 2018). Alternatively, the closing, or narrowing, of certain preservational pathways and windows has also been hypothesised to result in an apparent absence of Ediacaran taxa from Phanerozoic deposits – the so-called Cheshire cat model (Laflamme *et al.* 2013) – but this is not favoured (Laflamme *et al.* 2013; Wood *et al.* 2019) because of the persistence of Proterozoic mat-ground environments well into the Cambrian Period. In order to improve our understanding of the

end-Ediacaran, we require more refined estimates of the timing of significant geochemical events and an improved understanding of ecosystem interactions in the terminal-Ediacaran.

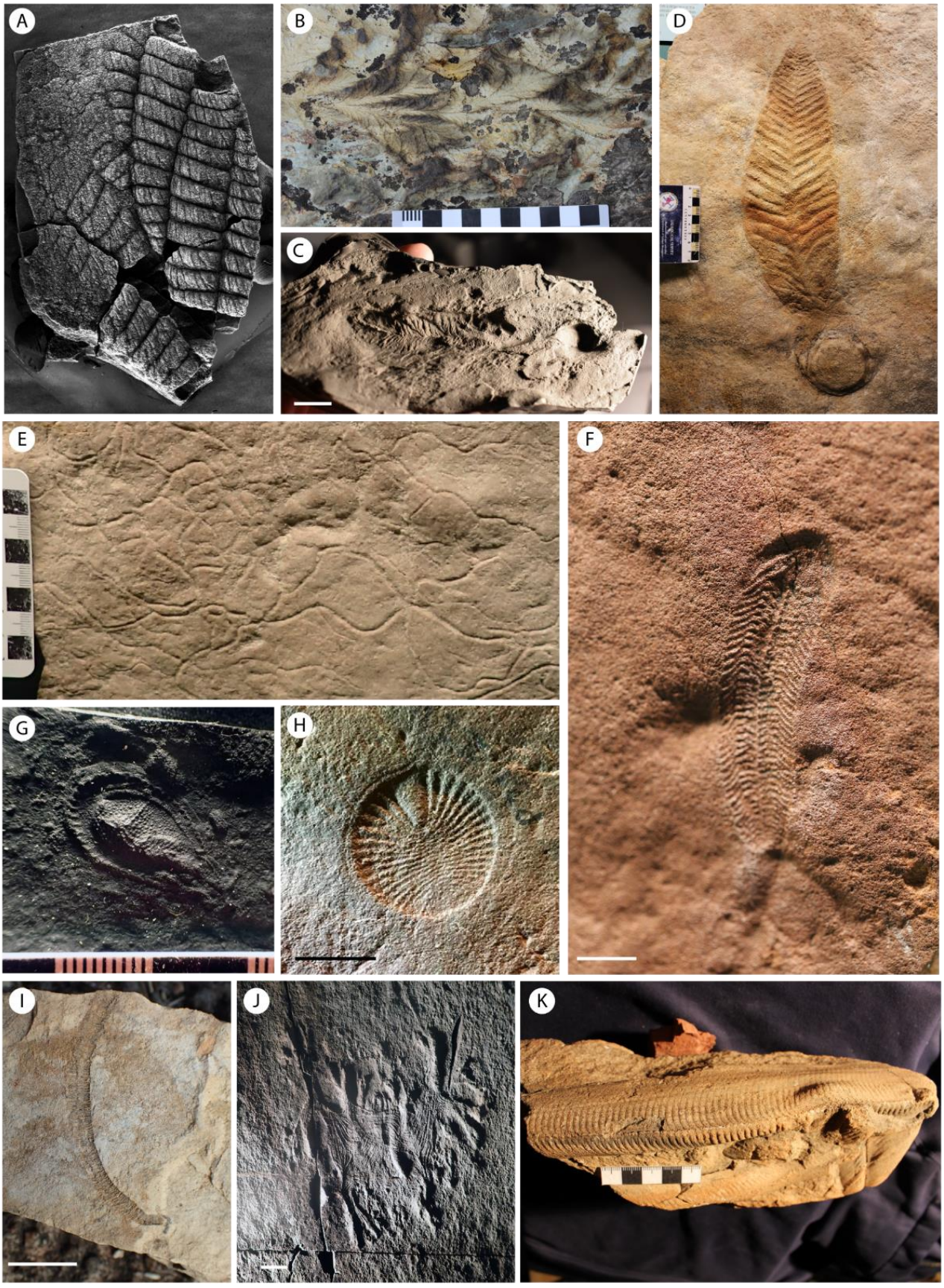
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**Figure 1.2:** The disparity of Ediacaran macrofossil form. **A)** *Charnia masoni* from Russia (PIN 3993 – 7018), **B)** *Bradgatia* sp., specimen remains in field, from Newfoundland **C)** *Trepassia wardae* from Newfoundland **D)** *Arborea arborea* from Australia (SAM P19690a), **E)** *Helminthoidichnites* isp. From Australia (SAM P42142), **F)** *Spriggina floundersi* from Australia (SAM P40137), **G)** *Kimberella quadrata* from Russia (ROM 62392), **H)** *Dickinsonia costata* from Australia (SAM P34224), **I)** *Corumbella wernerii*, specimen remains in field, from Brazil **J)** *Haootia quadriformis* from Newfoundland (holotype housed at The Rooms Provincial Museum, St. Johns), **K)** *Pteridinium simplex* from Namibia. Image provided by M. Laflamme.

## 1.3 What's in a name?

Individually and collectively, members of the Ediacaran Macrobiota have been both allied with extant clades (e.g. Glaessner 1984), and deliberately set apart from them by suggestions that they were either 'failed experiments' in the history of life, or members of long-extinct higher-order clades (e.g. Shen *et al.* 2008). Despite their undoubted value in stimulating debate around Ediacaran taxa, I argue that these latter viewpoints have hampered Ediacaran research. They have also created confusion within the wider community as to the placement of the Ediacaran Macrobiota in the tree of life, forming a barrier to their incorporation within biological and developmental discussions. I advocate abandoning the failed experiment perspective, and embracing phylogenetic thinking in order to make progress in determining the phylogenetic positions of these organisms, and realising their evolutionary significance.

### 1.3.1 The development of Ediacaran taxonomic terminology

As taxa were formally described throughout the 1960s and 1970s, members of the Ediacaran biota were frequently considered to belong to derived animal clades including the pennatulacean cnidarians (Glaessner and Wade 1966) and the annelid worms (Wade 1972), although non-metazoan opinions were occasionally expressed. During the 1970s and 1980s, these hypotheses were challenged by a school of thought that sought to remove the Ediacaran biota from extant higher-order groupings and place them within new phyla or kingdoms. Phylum Petalonamae (Pflug 1972) ('Nama petals', named by Hans Pflug after fossils

described from the Nama desert of Namibia) included several frondose taxa (e.g. *Charnia masoni*) and was initially considered to represent ancient animals, but was later revised to lie intermediate to the animal and plant Kingdoms and distinct from all living forms (Pflug 1973). Pflug used the term Petalo-organisms to describe a grade of organisation, including the Petalonamae (with an anatomy closer to animals than plants) and Petalostromae (with an anatomy closer to plants than animals) (Pflug 1973) (Fig. 1.3D). A recent study has placed Phylum Petalonamae as sister to the Eumetazoa, but includes a broader suite of taxa than were originally recognised by Pflug (Hoyal Cuthill and Han 2018). Phylum Proarticulata (Fedonkin 1983, 1985) included taxa such as *Dickinsonia,* and was considered to comprise a clade of bilaterian animals with similarities to the (now defunct) Superphylum 'Articulata' (e.g. Edgecombe 2009). Mikhail Fedonkin envisaged a situation in which truly segmented animals (Articulata) evolved from Ediacaran organisms with an offset form of bilateral symmetry, which themselves derived from radially symmetrical ancestors he considered related to jellyfish (Fedonkin 1985) (Fig. 1.3B). The radical Vendobiont hypothesis of Adolf Seilacher (Seilacher 1989; 1992) united all Ediacaran taxa that displayed a 'serially quilted' anatomy (including those listed above) within Kingdom Vendobionta (meaning 'Vendian Life' after the Russian stratigraphic term for the latest Precambrian), on the basis of taphonomic and constructional arguments. Seilacher and colleagues later modified these views, first revising Kingdom Vendobionta to an extinct sister-Phylum to the Eumetazoa (Buss and Seilacher 1994), before considering vendobionts as an extinct Class (Seilacher *et al.* 2003) or Subclass (Seilacher 2007) of giant protists (Fig. 1.3C). This repeated revision may have been due to Seilacher's recognition that an extinct higher order clade was an unsatisfactory solution (Buss and Seilacher 1994, Seilacher *et al.* 2003) to the Ediacaran problem. Despite the radical nature of these hypotheses, they initially attracted considerable support, although questions were raised as to whether there was sufficient evidence to support the Vendobiont hypothesis, with alternative suggestions that the Ediacaran biota may simply represent a subset of Neoproterozoic biodiversity (Runnegar 1995).

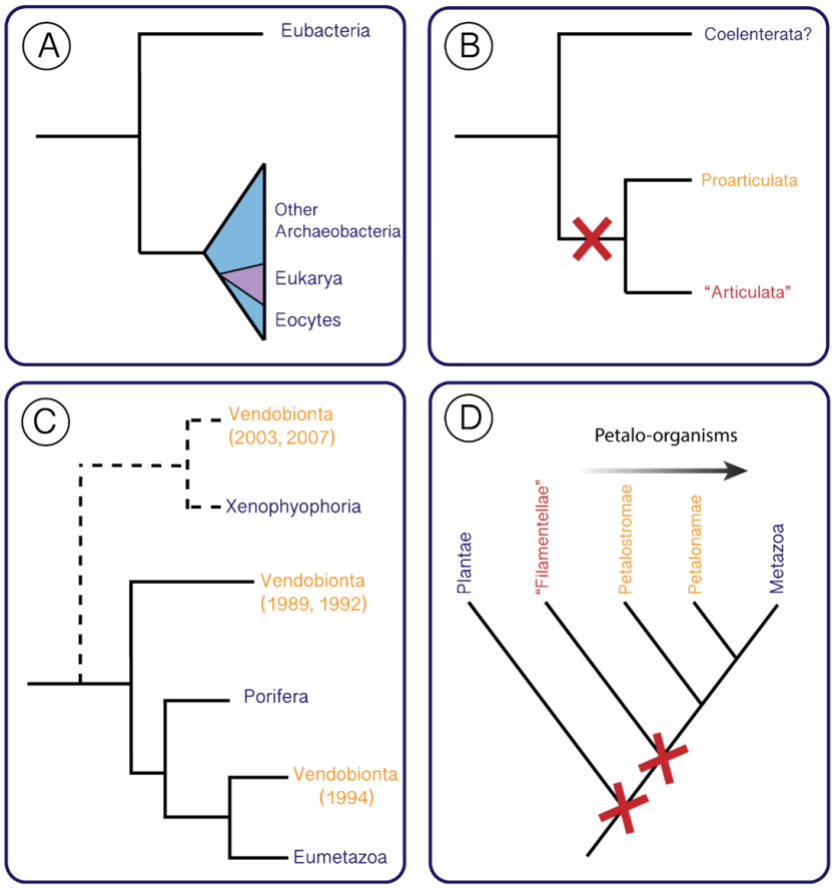


**Figure 1.3:** How cladistic thinking affects our view of the Ediacaran Macrobiota. Crosses indicate nodes that are no longer supported, orange labels are proposed Ediacaran clades, red labels indicate clades that are now defunct. **A)** Molecular phylogenies predict a single origin of life, and therefore all organisms must fall within the known tree of life (Williams *et al.* 2013). **B)** The Proarticulata (as originally defined) cannot be reconciled with modern phylogenetic thinking, since the group to which it was most closely allied – the Articulata – has been rejected (e.g. Edgecombe 2009). **C)** Previously hypothesised higher-order positions for the Vendobionta ultimately resolve them as either stem-group metazoans or eumetazoans (Seilacher 1989, 1992; Buss and Seilacher 1994), which remain viable phylogenetic hypotheses, or later as protists similar to xenophyophores (Seilacher *et al.* 2003; Seilacher 2007) (dates indicated). **D)** The petalo-organisms, as conceived by Pflug, represent a grade of organisation between the animals and the plants, encompassing the Petalonamae and Petalostromae, which he interpreted as clades. Molecular phylogenetics has since demonstrated that the animal and plant Kingdoms are not sister clades (Baldauf and Palmer 1993).

### 1.3.2 Breaking up the Ediacaran biota

In challenging earlier metazoan interpretations, the hypotheses of the 1970s and 1980s brought the Ediacaran Macrobiota to the attention of the wider scientific community, and laid the foundations for subsequent debate on their phylogenetic placement. A wealth of anatomical, palaeoecological and developmental specimen data has resulted from these investigations, and the majority of scientists now consider the Ediacaran Macrobiota to be a polyphyletic grouping (Ivantsov and Malakhovskaya 2002; Xiao and Laflamme 2009). Some taxa are reasonably considered to be candidate metazoans, most notably *Dickinsonia*, whose metazoan placement is now supported by developmental and ichnological studies, while biomarker data supports a holozoan placement (Ivantsov and Malakhovskaya 2002; Gold *et al.* 2015; Hoekzema *et al.* 2017; Bobrovskiy *et al.* 2018), whereas others are allied with non-metazoan eukaryotic groups, such as the protistan-grade *Palaeopascichnus linearis* (Kolesnikov *et al.* 2018), or *Beltanelliformis* – interpreted as a cyanobacterial colony (Bobrovskiy *et al.* 2017). Today, as consensus tends away from a clade of the Ediacaran Macrobiota, undermining the previously defined higher-order groupings (though with some exceptions, e.g. Hoyal Cuthill and Han 2018), many researchers continue to cite Seilacher, Pflug and Fedonkin's works as a mechanism to demonstrate the idiosyncratic nature of these fossils. There is a particular tendency to cite only Seilacher's early work, asserting his idea that the Ediacaran Macrobiota could represent an extinct Kingdom as a viable hypothesis, while often neglecting his later publications and taxonomic revisions, and overlooking the fact that he himself acknowledged the presence of metazoans (in the form of ichnofossils, and macrofossils like *Kimberella quadrata*) amongst Ediacaran fossil assemblages (Seilacher 1989, 1992; Buss and Seilacher 1994; Seilacher *et al.* 2003). Several studies also continue to advance the view that the Ediacaran Macrobiota are failed experiments by virtue of being extinct and not possessing known direct (living) descendants (e.g. Shen *et al.* 2008; Bamforth and Narbonne 2009). I argue that continued consideration of this viewpoint is detrimental to efforts to advance knowledge of Ediacaran Macrobiota taxa.

### 1.3.3 Weird wonders or extinct ancestors?

Macrofossils from the Ediacaran Period may well be strange, and many taxa remain difficult to interpret, but they can, and should, be interpreted within the framework of phylogenetic

thinking (Runnegar 1995). Subjecting problematic fossils to hypotheses that displace them from extant groupings is not uncommon, as exemplified by the Cambrian organisms of the Burgess Shale. Stephen Jay Gould, amongst others, noted the unique anatomies of many fossils of the Burgess Shale, which he did not recognise in any extant animal phyla. Notably, rather than viewing these 'weird wonders' as failed experiments, Gould recognised the significance of the strange Cambrian taxa, some of which he did not consider to sit within any known animal phyla "of this or any former Earth" (Gould 1989, p. 134). Furthermore, study of these unusual organisms has gradually resolved their relations to extant clades, as application of the stem and crown group concept permeated invertebrate palaeontological research (Brysse 2008).

If we accept that life has a single origin (Fig. 1.3A), Ediacaran macrofossils must occupy a branch/branches in the known tree of life. Any phylogenetic framework must reflect contemporary knowledge, such that proposed Ediacaran clades are grounded in current phylogenetic consensus. For example, the Proarticulata were considered ancestors to the Articulata, a group that molecular data now suggest to be invalid (e.g. Edgecombe 2009), with metazoan segmentation evolving independently at least three times (Chipman 2010). Only when the correct phylogenetic position(s) for the Ediacaran Macrobiota has been established can their evolutionary success or failure be assessed (Fig. 1.3). Evolutionary success can be measured in many ways, and does not necessarily correlate with survivorship: were trilobites an evolutionary failure? If these organisms are resolved as being either paraphyletic, or a polyphyletic assemblage (Xiao and Laflamme 2009; Erwin *et al.* 2011; Grazhdankin 2014; Tarhan *et al.* 2018), it would be inappropriate to consider them a failed experiment, and in time we may consider some of the characters they possess (such as axial arrangement) as homologous with those of extant taxa. We recognise that at least some Ediacaran taxa were members of groups with extant representatives, including both metazoan (e.g. *Dickinsonia*) and non-metazoan clades (e.g. *Palaeopascichnus*). They persisted for 30 million years, with evidence for considerable diversification, and they display the capacity to form complex ecosystems (Mitchell and Butterfield 2018; Darroch, Laflamme and Wagner 2018). At their zenith, the Ediacaran Macrobiota were arguably hugely successful, but we cannot rationalise any of these observations until the ultimate positions of the Ediacaran Macrobiota in phylogeny are known.

### 1.3.4 Moving forward

As the relatively young field of Ediacaran research continues to make rapid advances, I propose that Ediacaran macrofossil taxa should be considered on a case-by-case basis, with no underlying assumption of monophyly of the Ediacaran Macrobiota. I advocate moving away from the use of leading terminology, which either deliberately divorces members of the Ediacaran Macrobiota from living taxa, or asserts unproven relationships.

Assertion of whether or not the Ediacaran Macrobiota represent failed experiments is premature while their phylogenetic positions remain unknown. We must also remember that if we are to fully appreciate the information these taxa can provide, we should define them not only by what sets them apart, but also by similarities to living forms. Future work detailing specific hypotheses of affinity should be based on positive evidence wherever possible, and be grounded in phylogenetic systematics, with careful consideration of a broad suite of characters, integrating across anatomical, developmental and reproductive data, and recognising the impact of different taphonomic regimes.

Holistic combination of such biological and palaeontological data offers our best route towards elucidating the early history of complex macroscopic organisms. The Ediacaran Macrobiota must be restored to the known tree of life.

## 1.4 Evolutionary – Developmental biology

Evolution by natural selection and, indeed, genetic drift, acts on variation that exists in populations. Variation may be introduced in many ways, but a common mechanism is through changes in the developmental programme of an organism (e.g. Moczek 2008). Therefore, establishing which aspects of growth are conserved, and which are variable (and how they are variable) between different clades allows inference to be made about the generation of morphological disparity. By studying patterns in development, we may better understand enigmatic fossil groups – like members of the Ediacaran Macrobiota, whose anatomy is not

easily reconcilable with extant clades – where the generation of form can act as the homology, rather than the form itself (e.g. Chipman 2010).

The past 30 years has witnessed the emergence of the field of Evolutionary-Developmental Biology, which extends the principles of the Modern Synthesis of Evolution to include the study of ontogeny. The significance of ontogeny was not lost on the pioneers of the Modern Synthesis; Julian Huxley acknowledged this in his seminal text 'Evolution: The Modern Synthesis': "… *a study of the effects of genes during development is as essential for an understanding of evolution as are the study of mutation and that of selection*." Recently developmental mechanisms have been recognised as unique loci of innovation upon which selection may act (e.g. Müller 2007), and as such carry significant weight in discussion of the origination and diversification of morphological variation.

Homeotic genes are known to regulate the generation of anatomy, and as such have been widely invoked in the generation of morphological novelty (e.g. Akam, Dawson and Tear 1988; Carroll 1995; Di-Poi *et al.* 2010). These links were robustly demonstrated during the late 1970s and 1980s, with the rise of molecular developmental genetics. Both animal and plant biologists began the study of homeotic mutants, and illustrated deep homologies intrinsically linked to the evolution of complexity, with the generation of *D. melanogaster* with the *Antp* (Antennapedia) mutant gene, causing the fly to develop ectopic legs in place of antennae (Lewis 1978; Nüsslein-Volhard and Wieschaus 1980). *Antp* belongs to a group of genes called the HOX genes.

HOX genes are a set of transcription factors which act to specify anterior-posterior patterning in bilaterian animals, and the oral-aboral axis in cnidarians. As such, they play a master role in establishing the body plan during embryogenesis. Many researchers have proposed a link between segmental duplication of individual homeobox genes, or gene clusters in gene regulartory networks and the generation of evolutionary novelty (e.g. Brooke, Garcia-Fernandez and Holland 1998; Davidson and Erwin 2006; Soshnikova *et al.* 2013; Holland 2015), and the 'Cambrian Explosion' of animal diversity (e.g. Davidson and Erwin 2006; Holland 2015). It is often reported that the establishment of developmental networks, by the cooption of homeotic genes played a crucial role in establishing characters required for the

radiation of bilaterian body plans (e.g. a through gut; Cavalier-Smith 2006; Holland 2015). As animal genomes are fundamentally comparable, researchers have moved away from explaining the Cambrian radiation through the acquisition of many new and important genes, and now associate the diversification of bilaterian bodyplans with the cooption of pre-existing genes into new gene regulatory networks (e.g. Davidson and Erwin 2006). This may have increased developmental morphospace. There remains debate concerning the evolution of developmental morphospace, and how different facets like the cannalisation of developmental pathways (Valentine 1995), perhaps enhanced by an expanding miRNA complement were involved in the 'fixing' of the animal phyla (discussed in Peterson, Dietrich and McPeek 2009).

Similar advances were being made in understanding plant biology in the late 1980s and early 1990s, where it was shown that organ identity was specified by a set of common factors across distantly related species (Coen and Meyerowitz 1990). These factors were later identified as a set of transcription factors belonging to the MADS-box family, and some are considered to play a major role in the ABC model of floral development (Coen and Meyerowitz 1991), where different organs are specified by different combinations of homeotic genes (e.g. *AGAMOUS* – a C function floral identity gene, involved in stamen and carpel identity). MADS-box family genes are found across the plants, animals and fungi, but appear to have undergone several rounds of duplication in the angiosperms, and these underlying polyploidy events are often linked to the diversification of this clade (reviewed in Soltis *et al.* 2009).

This work was crucial both in highlighting changes in development as a basis for the creation of diversity, disparity and complexity, and in illustrating the commonality of developmental process and pattern. In the years since, much work has sought to explain the evolution of morphological novelty as 'variation on a theme', i.e. in HOX gene duplication, cooption or deletion, as detailed above (e.g. Glassford *et al.* 2015; Setton *et al.* 2018). For example, while segmentation proceeds differently across the segmented bilaterian phyla – with for example mesodermal somites in vertebrates, and ectodermal segments in arthropods – they have evolved from common pathways in axis specification and anterior-posterior patterning, including cyclical Notch and Wnt (Chipman 2010). It is the generation of form that is the

homology, not the form itself, validating the study of development in establishing a suite of characters with which we might compare enigmatic fossil groups to living clades.

## 1.5 Thesis outline

The aim of this thesis is to provide developmental interpretations for a number of enigmatic Ediacaran macrofossils in order to better understand their potential affinities. I consider members of the soft-bodied Ediacaran Macrobiota over other putative Precambrian animals because they are known from many specimens and often across a large size range, which allows for populations of individual taxa to be examined. While members of the Ediacaran Macrobiota are morphologically diverse, I focus largely on the frondose members of the biota – rangeomorphs and arboreomorphs. This is because although often considered as animals today, (e.g. Budd and Jensen 2017; Darroch *et al.* 2018) these groups have historically been subject to the most disparate and largest number of phylogenetic interpretations, and are the most ancient. In addition, the frondose component of the Ediacaran Macrobiota are amongst the best studied from other perspectives (e.g. ecological or reproductive [Darroch *et al.* 2013; Mitchell *et al.* 2015; Kenchington and Wilby 2017; Mitchell and Butterfield 2018; Mitchell and Kenchington 2018]), and so there is more information to aid interpretation than with other, less studied components of the biota.

Chapter two seeks to incorporate all previously published developmental and anatomical data on a subset of Ediacaran taxa into a new synthesis of growth and development of three well-known Ediacaran morphogroups: the Rangeomorpha, the Dickinsoniomorpha and the Erniettomorpha. I then consider this evidence in light of what is known of patterns of morphogenesis in extant serially-repetitive taxa, and thus constrain where these different morphogroups may sit in eukaryotic phylogeny. This provides a hypothesis of affinity against which newly collected data (presented in later chapters) can be assessed.

Chapter three is an anatomical and developmental reassessment of the enigmatic South Australian frondose fossil *Arborea arborea* (after which the morphogroup Arboreomorpha is named). This morphogroup is not discussed in Chapter two because of systematic

uncertainty. Nonetheless, arboreomorphs remain an important component of the Ediacaran Macrobiota. *A. arborea* is known largely from fragmentary remains, however, I recognise a number of new anatomical characters that allow for construction of a new model of anatomy and development. I also describe a number of potential specimens of *A. arborea* from Charnwood Forest (~562–557 Ma), extending the stratigraphic range and environmental tolerances of this organism.

Chapter four constitutes a reassessment of the anatomy of *Charnia masoni* from three populations of specimens from different global localities (UK, Canada and Russia). This study represents the first attempt to integrate anatomical information from multiple global sites and (as best we can) across taphonomic regimes and populations biases. This study is undertaken under the assumption that a detailed understanding of anatomy is prerequisite to any study of growth, development or phylogenetic affinity. It reveals a number of uncertainties in our understanding of what is one of the best studied Ediacaran macrofossils, and proposes a new model of anatomy for the organism. I consider this against rangeomorph taxonomic and diagnostic criteria, and conclude that certain features of rangeomorph taxonomic schemes are inappropriate.

Chapter five builds on chapter four by presenting an analysis of the growth and development of *Charnia masoni.* This is assessed using population analysis to derive a new model of development from differentially sized specimens, as well as tomographic data from new three-dimensionally preserved specimens of *C. masoni* from the Lyamtsa Formation, White Sea, Russia. I produce a new synthesis of growth and development in *C. masoni* evidencing a programme of growth far more complex than any previously invoked for this taxon, and a pattern of branching morphogenesis that is unlike any branching organism alive today. Finally, I bring this data together in a new phylogenetic hypothesis for *C. masoni*.

Chapter six brings together the key results from chapters two to five in a discussion of the evolutionary implications of my data on our understanding of metazoan evolutionary and developmental dynamics at this time. I also propose a programme of future work, which aims to expand the work and techniques used in this thesis in order to better understand the rise of the animals.

# Chapter 2:

## Ediacaran Developmental Biology:

**Author contributions:** A version of this chapter is published in *Biological Reviews.*

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F.S.D, A.G.L and P.C.J.D designed the study, F.S.D conducted all analyses and produced the first draft of the manuscript. All authors provided feedback on the manuscript. F.S.D is lead author of the paper, and contributed ~85% of the work presented in this chapter.

# Abstract:

Rocks of the Ediacaran System (635–541 Ma) contain fossil evidence for some of the earliest complex macroscopic organisms, many of which have been interpreted as early animals. However, the unusual morphologies of some of these organisms have made it difficult to resolve their biological relationships to modern metazoan groups. If a metazoan affinity can be demonstrated for these organisms, as advocated by many researchers, they could prove informative in debates concerning the evolution of the metazoan body axis, the making and breaking of axial symmetries, and the appearance of a metameric body plan. Attempts to decipher members of the enigmatic Ediacaran Macrobiota have largely involved study of morphology: comparative analysis of their developmental phases has received little attention. Here I present what is known of ontogeny across the three iconic Ediacaran taxa *Charnia masoni*, *Dickinsonia costata* and *Pteridinium simplex*, together with new ontogenetic data and insights. I use these data and interpretations to re-evaluate the phylogenetic position of the broader Ediacaran morphogroups to which these taxa are considered to belong (rangeomorphs, dickinsoniomorphs and erniettomorphs), and conclude, based on the available evidence, that the affinities of the rangeomorphs and the dickinsoniomorphs lie within Metazoa.

## 2.1 Introduction

Among multicellular eukaryotes, Metazoa are unique in exploring a broad range of diverse body plans. Assisted by their ability to undergo coordinated embryogenesis (Valentine, Tiffney and Sepkowski, 1991), and free from the restrictions of rigid cell walls, animals have evolved well over 100 distinct cell types [compared to ~7 in fungi and kelps and ~30 in higher plants (Bonner, 1988)], and have arranged them into diverse tissue types, physiological systems, and morphological structures. Animals are therefore among the most biologically complex organisms. Elucidating the developmental processes that underpin this complexity represents a major challenge in contemporary evolutionary and developmental biology.

It is perhaps surprising that although developmental insights can be gleaned from Ediacaran fossil assemblages, Ediacaran developmental biology remains in its infancy. The little work that has been done, based on the premise that ontogenetic characters are considered to have been conserved across evolutionary time, demonstrates the potential power of morphogenesis in testing established hypotheses of affinity (e.g. Antcliffe and Brasier, 2007: Gold *et al.*, 2015). Investigation of morphogenesis in Ediacaran taxa also has the potential to constrain hypotheses of developmental evolution associated with the evolutionary emergence of animals, and to test models of trait evolution that are currently based only on theoretical predictions. Here I review the existing data and interpretations regarding morphogenesis in key Ediacaran macro-organisms, and use this information to constrain hypotheses of their evolutionary relationships to extant eukaryotic groups.

## 2.2 The semantics of Ediacaran morphogenesis

Describing ontogeny in fossil organisms can be problematic (e.g. Hone, Farke and Wedel, 2016). Many extant organisms display some form of ontogenetic shift (Paris and Laudet, 2008) and this is often used to distinguish between juvenile and adult individuals. However, such shifts are difficult to identify with certainty in extinct organisms, and have typically not been

recognised in Ediacaran fossil taxa, whose adult and juvenile stages have largely been distinguished based only on the size of the specimens (e.g. Liu *et al.*, 2012). Moreover, many extant clades, including several metazoan groups to which members of the Ediacaran Macrobiota have been compared, exhibit a morphologically distinct juvenile stage that bears little resemblance to the adult form (e.g. the planula larvae of Cnidaria). Discrimination of adults and juveniles among Ediacaran macrofossils is not, therefore, something that we can necessarily expect to achieve, and such terms should be avoided. The alternative use of 'size classes' is both arbitrary and potentially subject to change as new specimens are described. Allocation of specimens to 'generations' is another possibility (see Mitchell *et al.* 2015), but at least some bedding-plane assemblages of Ediacaran macro-organisms are considered to reflect only single generations, despite large variance in size (Darroch, Laflamme and Clapham, 2013; although see Wilby, Kenchington and Wilby, 2015). The simplest and most defensible strategy is to consider how the size of a fossil relates to smaller and larger specimens of the same species, and to make the reasonable assumption that larger individuals would have been older, or at least more developed, than smaller individuals (see Fedonkin, 2002; Narbonne, 2004; Flude and Narbonne, 2008).

Understanding the difference between pattern and process is also essential when considering growth in fossil taxa. It is clear that many Ediacaran taxa were composed of multiple units, which have at various points been termed branches, modules, units, isomers or segments. All the taxa that I address have been considered to grow either by inflation (wherein a particular unit increases in size), 'insertion' (the sequential addition of units to an organism), or a combination of these (see Table 2.1 for a comparison of the distribution of these strategies across published Ediacaran taxa). However, process terms must have a basis in ontological data (Jardine 1969) and inferences of process should be evidenced and rationalised from assemblages of individuals representing different developmental stages. New structures and units can be added during the development of multicellular organisms in a variety of patterns, but this invariably occurs through differentiation of existing cells and tissues. Insertion of units, in the sense that it is described in Ediacaran macro-organisms, does not occur in development, except in a metaphorical sense. Unfortunately, the metaphorical concept of unit insertion is at risk of being reified as a literal process in the interpretation of these fossils. Thus, I recommend use of the term 'differentiation' in place of 'insertion'. This ensures that I

do not limit comparisons to only those extant taxa that show *de novo* addition of new units. I use the term 'insertion' when summarising previous developmental studies of Ediacaran taxa in the following sections, but then revert to use of 'differentiation' from section 2.4 onwards.

Finally, I note that previous rangeomorph taxonomic schemes have focused on assumed polarity of growth, considering various organisms as either unipolar, bipolar or multipolar (Brasier, Antcliffe and Liu, 2012). However, the assumption that growth is occurring in the positions ascribed by these terms remains untested in many rangeomorphs. I prefer here to use morphologically descriptive terminology (as opposed to morphogenetically descriptive). Previous attempts at morphological description have considered fronds to be constructed of petalodia and petaloids (Laflamme and Narbonne, 2008), but such terminology has more recently been considered inappropriate, since its correct deployment is also somewhat reliant on a complete understanding of an organism's life history (Brasier *et al*., 2012). I therefore introduce the terms 'uniterminal', 'biterminal' and 'multiterminal' as morphological descriptors of the number of distal tips the frond possesses (not including the stem or holdfast). In practical application, previous groupings of rangeomorphs remain the same, but the new terms here are based entirely on morphological features, and avoid all assumptions regarding morphogenesis.

| Morphotype | Taxon | Inflation | Differentiation | Reference |
|---|---|---|---|---|
| Rangeomorph | *Charnia masoni* | Allometric | Observed | Brasier *et al*. (2012); Antcliffe and Brasier (2008) |
| Rangeomorph | *Vinlandia antecedens* | | | |
| Rangeomorph | *Trepassia wardae* | Minimal | Observed | Narbonne *et al*. (2009) |
| Rangeomorph | *Beothukis/Culmofrons plumosa* | Present | Not-observed | Laflamme *et al*. (2012); Liu *et al*. (2016) |
| Rangeomorph | *Beothukis mistakensis* | Allometric | Not-observed | Laflamme *et al*. (2012); Liu *et al*. (2016) |
| Rangeomorph | *Avalofractus abaculus* | | | |
| Rangeomorph | *Fractofusus andersoni* | Isometric | Not-observed | Darroch *et al*. (2013); Gehling & Narbonne (2007) |
| Rangeomorph | *Fractofusus misrai* | Allometric/ Isometric | Not-observed | Darroch *et al*. (2013); Gehling & Narbonne (2007) |
| Rangeomorph | *Bradgatia linfordensis* | | | |
| Rangeomorph | *Bradgatia* sp. | Present | Not-observed | Flude & Narbonne (2008) |
| Rangeomorph | *Primocandelabrum hiemaloranum* | | | |
| Rangeomorph | *Primocandelabrum* sp. | | | |
| Rangeomorph | *Hapsidophyllas flexibilis* | | | |
| Rangeomorph | *Frondophyllas grandis* | | | |
| Rangeomorph | *Plumeropriscum hofmanni* | | | |
| Rangeomorph | *Pectinifrons abyssalis* | Present | Observed | Bamforth *et al*. (2008) |
| Dickinsoniomorph | *Andiva ivantsovi* | Isometric | | Fedonkin (2002) |
| Dickinsoniomorph | *Dickinsonia costata* | Allometric | Observed | Hoekzema *et al*. (2017); Evans *et al*. (2017); Gold *et al*. (2015); Ivantsov (2007); Retallack (2007); Runnegar (1982) |
| Dickinsoniomorph | *Dickinsonia lissa* | Present | | Ivantsov (2007) |
| Dickinsoniomorph | *Dickinsonia rex* | Present | Observed | Ivantsov (2007); Retallack (2007) |
| Dickinsoniomorph | *Dickinsonia tenuis* | Present | Observed | Ivantsov (2007); Retallack (2007) |
| Dickinsoniomorph | *Windermeria aitkeni* | | | |
| Dickinsoniomorph | *Yorgia waggoneri* | | Observed | Ivantsov (2007) |
| Erniettomorph | *Ernietta plateauensis* | Present | Not observed | Ivantsov *et al*. (2016); Dzik (1999) |
| Erniettomorph | *Nasepia altae* | | | |
| Erniettomorph | *Palaeoplatoda segmentata* | | | |
| Erniettomorph | *Phyllozoon hanseni* | | | |
| Erniettomorph | *Pteridinium simplex* | Present | Observed | Grazhdankin & Seilacher (2002) |
| Erniettomorph | *Swartpuntia germsi* | | | |
| Erniettomorph | *Valdainia plumosa* | | | |

**Table 2.1**. Summary of inflationary and 'insertional' (here renamed 'differentiation', see section 2.2 for details) styles of growth across taxa belonging to the Ediacaran morphogroups Rangeomorpha, Dickinsoniomorpha and Erniettomorpha (*sensu* Erwin *et al.* 2011). Inflation is documented as minimal (if the organism is considered to grow almost exclusively by 'insertion'), allometric (if units of the organism appear to inflate at different rates or to different degrees), isometric (if units of the organism appear to inflate at a constant rate relative to one another, maintaining overall shape), or simply present (if not further information on the degree of inflation is given). Differentiation ('insertion') is either noted as observed or not-observed. Empty cells represent the absence of previously published data.

## 2.3 Ontogeny in Ediacaran morphogroups

To date, ~200 Ediacaran macrofossil taxa have been described (Fedonkin *et al.*, 2007), and multiple attempts have been made to group these within sub-groups of closely related organisms. Initially, many Ediacaran taxa were considered members of extant animal clades (e.g. Glaessner, 1984), but more recently they have instead been grouped according to their morphological similarity (Erwin *et al.*, 2011; Grazhdankin, 2014), with such groupings representing grades (rather than clades) of organism. I focus this study on fossils considered to belong to three widely recognised morphogroups that together include many of the most contentious members of the Ediacaran biota: the rangeomorphs, dickinsoniomorphs and erniettomorphs. Members of these groups have all, at some point, been interpreted as animals, with some researchers considering members of all three groups to share a self-similar body plan, perhaps indicating a common evolutionary history (Seilacher, 1989, 1992; Buss and Seilacher, 1994; Dececchi *et al.* 2017; Hoyal Cuthill and Han 2018). I favour the use of morphogroups because it confers phylogenetic neutrality, but I note the possibility that unrelated taxa may be grouped together within such morphogroups, potentially obscuring phylogenetic signal. These concerns may be allayed by independent attempts to resolve the phylogenetic relationships among the Ediacaran grades that have provided some support for the biological reality of some morphogroups (Dececchi *et al*., 2017). While I acknowledge that the composition of these morphogroups may not be entirely coherent in phylogenetic terms, I consider them to provide a useful framework within which to sample the disparity of Ediacaran macro-organism body plans.

Hoyal Cuthill and Conway Morris (2017) have attempted to explain variation among Ediacaran frondose organisms as a consequence of ecophenotypism, produced in response to variation in nutrient levels in the water column across different palaeoenvironments. These authors conclude that characters that are typically considered discrete may be malleable across a nutrient gradient and so taxa which are distinguished from each other based on these characters may be ecophenotypic variants of the same taxa. This suggestion potentially introduces an alternative explanation for morphological variation that would otherwise be interpreted as taxonomic or ontogenetic. While I recognise the presence of some

ecophenotypic variation within Ediacaran assemblages, I note that population-level studies of frondose organisms continue to document discrete taxonomic variation (e.g. Kenchington and Wilby, 2017). Hoyal Cuthill and Conway Morris (2017) based their study on only three, anatomically discrete, specimens, representing taxa that are known to co-occur on bedding planes (Narbonne *et al.*, 2009), consistent with morphological variation existing within assemblages subject to similar palaeoenvironmental regimes. Previous studies have identified ecological tiering in Ediacaran rangeomorph communities (Ghisalberti *et al.* 2014), an expected facet of growth if community dynamics were governed by local nutrient concentrations (Hoyal Cuthill and Conway Morris 2017). However, recent work has found no support for this hypothesis, suggesting that increased dispersal distance as an explanation for height dynamics in these communities (Mitchell and Kenchington 2018). Until relationships between morphology and ambient nutrient levels can be demonstrated conclusively, I consider size variation within Ediacaran taxa to reflect ontogeny. However, as I recognise some ecophenotypic variation in populations, I do not consider morphometric measurements (e.g. length v width) to be a good indicator of ontogenetic change between populations (i.e. on different bedding planes).

### 2.3.1 Rangeomorpha

Rangeomorpha (Fig. 2.1) encompasses organisms that share a body plan comprising one or multiple fronds constructed of serially repeated, leaf-like, self-repeating branches (see supplementary online material [SOM] of Erwin *et al.*, 2011). Rangeomorphs were seemingly sessile organisms that lived in deep- and shallow-marine depositional environments, and are a stratigraphically long-ranging morphogroup, spanning the interval ~571–541 Ma (Boag *et al.* 2016; Pu *et al.*, 2016). Rangeomorphs can be uniterminal (with one apparent distal terminus: e.g. *Charnia masoni*), biterminal (e.g. *Fractofusus*) or multiterminal (e.g. *Bradgatia*), and the arrangement of their branches has been proposed as a basis for distinguishing between taxa (Narbonne *et al.*, 2009; Brasier *et al*., 2012). Morphogenesis has been considered most widely in the cosmopolitan taxon *Charnia masoni*, which possesses many features characteristic of rangeomorphs (Brasier and Antcliffe, 2004, 2009).

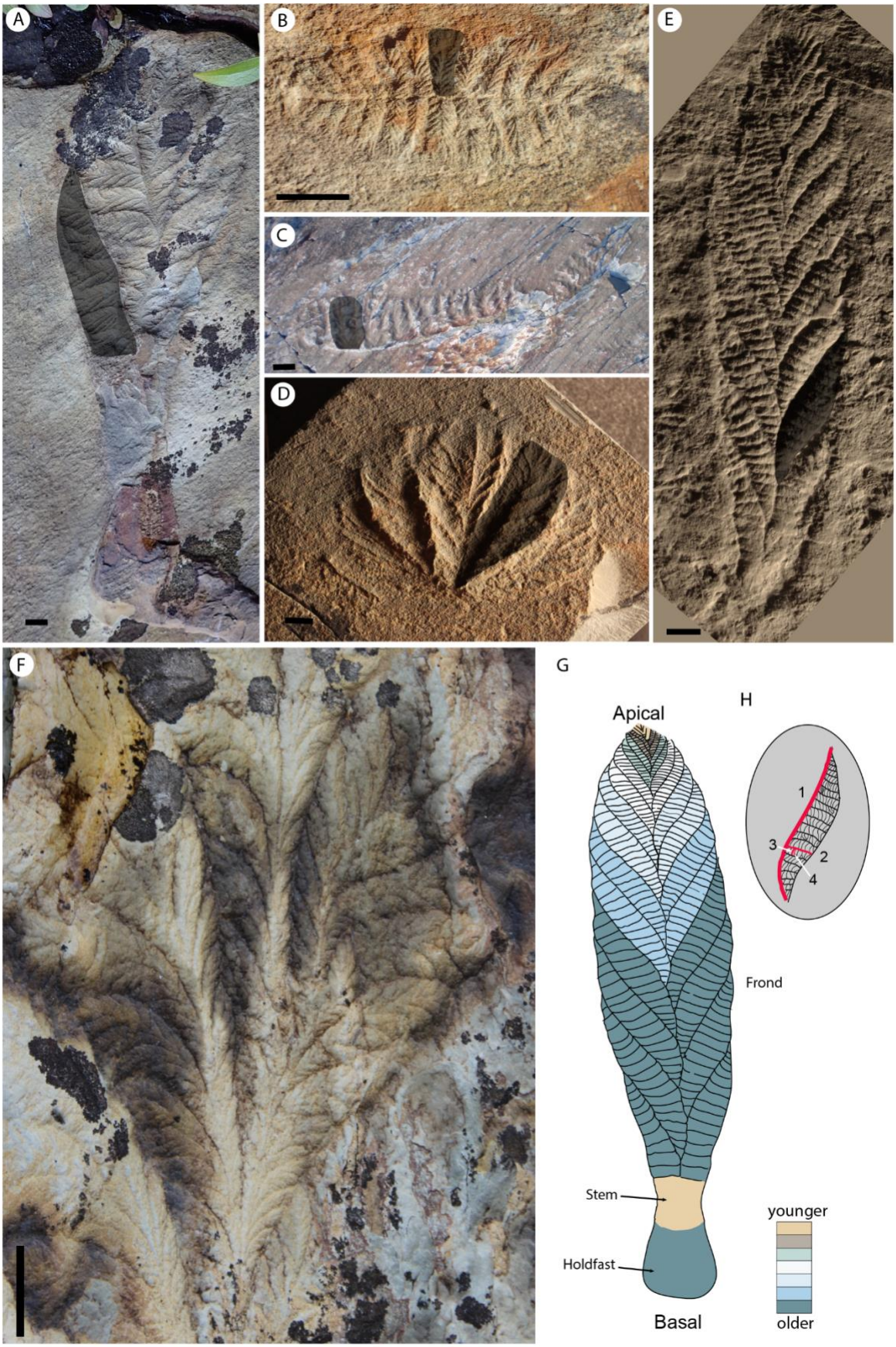
A
B
C
D
E
F
G
Apical
Frond
Stem
Holdfast
Basal
younger
older
H
1
2
3
4

**Figure 2.1.** Ediacaran rangeomorph taxa. **A)** *Beothukis plumosa*, Newfoundland, Canada. **B)** *Fractofusus andersoni*, Newfoundland, Canada. **C)** *Pectinifrons abyssalis,* Newfoundland, Canada. **D)** *Bradgatia* sp., Newfoundland Canada. **E)** *Charnia masoni*, UK. **F)** Higher-order branching in an exceptionally preserved *Bradgatia* sp., specimen from Newfoundland. **G)** Stylised interpretation of growth in first order branches in *Charnia masoni* based on specimen data presented in this chapter. **H)** The different orders of rangeomorph branches, and their arrangement within *Charnia masoni.* 1 = first order branch, 2 = second order branch, 3 = third order branch and 4 = fourth order branch. Grey overlay in A–E indicates a first order branch. Scale bars: A, B, D and E = 10mm, C = 5cm.

### *(a) Charnia masoni*

*Charnia masoni* (Fig. 2.1E) is a uniterminal rangeomorph with a global late Ediacaran distribution, found in the UK (e.g. Wilby *et al.*, 2015), Newfoundland (e.g. Laflamme *et al.*, 2007), northwestern Canada (Narbonne *et al.*, 2014), South Australia (e.g. Gehling and Droser, 2013), the White Sea of Russia (Fedonkin, 1990), and Siberia (e.g. Grazhdankin *et al.*, 2008). It has been variously compared to algae (Ford, 1958), fungi (Peterson, Waggoner and Hagadorn, 2003), stem-eumetazoans (Budd and Jensen, 2017), pennatulacean cnidarians (Glaessner, 1984), or placed in a hypothetical non-metazoan higher order group (Seilacher, 1989, 1992). Known *Charnia masoni* specimens range from ~1 cm (Liu *et al.*, 2012) to >65 cm (Boynton and Ford, 1995) in length, with size variants typically interpreted as different ontogenetic stages in the *Charnia* life cycle (e.g. Liu *et al.*, 2012).

*Charnia* individuals of all sizes share a similar gross morphology, possessing multiple first order branches lying at a high angle along a glide plane of symmetry running through the central axis of the frond. The smallest frondose specimens appear to lack a stem, but all are considered to possess a sediment-bound holdfast to anchor them to the seafloor (see fig 4b in Liu *et al.*, 2012). First order branches in the smallest specimens range from five in a specimen of 1.0 cm length to seven in a specimen of 1.3 cm (Liu *et al.*, 2012). Specimens longer than ~7 cm possess a clear but short stem, which can exhibit branching down its length (fig. 2b in Laflamme *et al.*, 2007; fig. 5.5 in Wilby *et al.*, 2015), thus distinguishing this feature from the discrete ‘naked’ stem (i.e. lacking branched subdivisions) of other rangeomorphs

(Laflamme, Flude and Narbonne, 2012) and non-rangeomorph frondose taxa (e.g. *Charniodiscus*; Laflamme, Narbonne and Anderson, 2004). There is a linear relationship between the number of first order branches in *Charnia masoni* and the overall length of the organism (Fig. 2.2), excepting the very largest specimens, which possess proportionally fewer branches than might be expected (Wilby *et al.*, 2015). First order branches increase in size as the organism increases in length (Wilby *et al.*, 2015). No specimens of *Charnia* have been observed to possess greater than four hierarchical orders of branching. Previously collected growth data are derived only from first order branches and so development in higher branching orders, and the number of branch orders in the smallest specimens, has yet to be discerned.

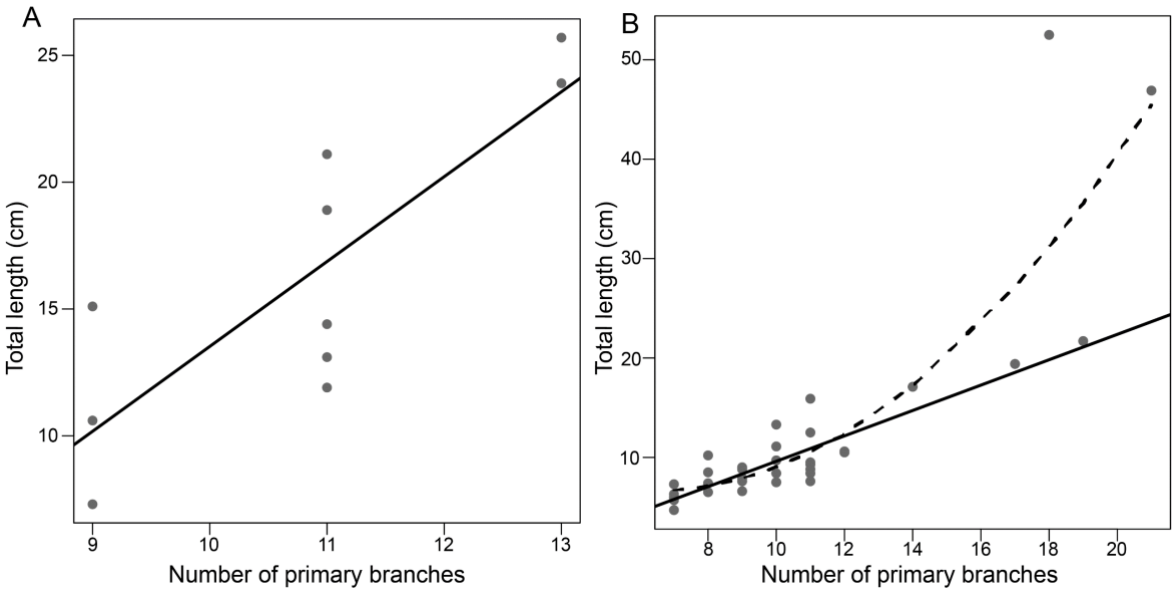


**Figure 2.2:** The length of *Charnia masoni* specimens plotted against the number of first order branches in specimens from: **A)** Sword Point, Newfoundland, Canada (data from Laflamme *et al.* 2007) (data have been retrodeformed); **B)** North Quarry Bed B, Charnwood Forest, Leicestershire, UK (data from Wilby *et al.* 2015) (data were not retrodeformed). Retrodeformation is the process by which specimen images are post-processed to remove for tectonic alteration. In the case of Ediacaran frondose fossils this entails changing the dimensions of holdfast structures to circular, on the assumption that this was their original shape. Linear models represented by solid line (fitted through a subset of data in B – excluding the two largest specimens); broken line represents a second-order polynomial model. Both populations show a linear relationship between specimen size and the number of first order

branches up to specimens 49cm in length [$P$= 0.006429 and $P$= 5.327 x $10^{-11}$ for the Laflamme *et al.* (2007) and Wilby *et al.* (2015) data sets, respectively]; specimens larger than this are not explained by a linear model [the complete Wilby *et al.* (2015) data set is best fitted by a second-order polynomial model, $P$= 1.579 x $10^{-10}$].

These previous observations have led to interpretation of *Charnia* as growing by the 'insertion' and subsequent inflation of branches (Wilby *et al.*, 2015). The consistent smaller size of first order branches at the apical region of individual fronds has been interpreted as evidence for a distal (apical) generative zone (Antcliffe and Brasier, 2007), with proximal first order branches (close to the holdfast) considered to have undergone a relatively longer inflation-driven period of growth (fig. 2 in Antcliffe and Brasier, 2007). The proportionally lower number of first order branches in the largest specimens could represent an ontogenetic shift from an initial 'insertion'-driven stage of growth to a second inflation-dominant interval with reduced rates of branch addition (Wilby *et al.*, 2015). The largest *Charnia* specimens have been proposed as evidence for indeterminate growth, and seem to show no upper size constraints (Wilby *et al.*, 2015).

The apparent absence of a stem in *Charnia* specimens less than ~7 cm in length may indicate that a stem was not present in the youngest organisms (Fig. 2.3A–B). It is possible that the stem and holdfast were buried in small specimens, lying beneath the plane of preservation. However, these smallest specimens exhibit a 'V'-shaped termination at their base, with no suggestion of any downwards extension of the basal branches (Fig. 6A–B). If the stem was truly absent in early ontogenetic stages, emerging only later in the life cycle, the notion of *Charnia* possessing a single, distal growth tip (*sensu* Antcliffe and Brasier, 2007) becomes questionable since growth would also have occurred in a generative zone at the proximal end of the organism (depicted in Fig. 2.1G). Although *Charnia* undoubtedly possessed its smallest first order branches in the distal region of the frond (Antcliffe and Brasier, 2007), this observation alone is not proof of a solitary, distal, growth tip (see also Hoekzema *et al*., 2017).

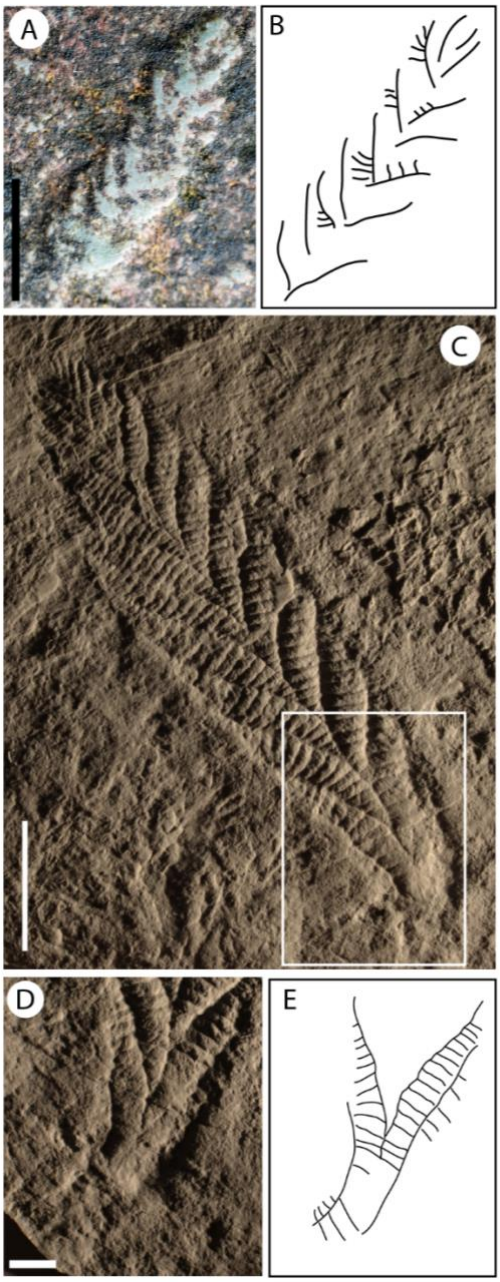


**Figure 2.3:** The development of the 'stem' region in *Charnia masoni*. **A, B)** *Charnia masoni* from Pigeon Cove, Mistaken Point Ecological Reserve, Newfoundland, Canada (**A**) and outline of specimen (**B**). **C)** *Charnia masoni* from Charnwood Forest, Leicestershire, UK. (**D**, **E**) Stem area (enlargement of boxed region in **C**) (**D**), and in outline (**E**). Illustrations do not include third (or higher) order sub-division. Scale bars: A = 5mm, C = 5cm, D = 10mm.

*(b) Ontogenetic trends across the rangeomorphs*

Interpretations of growth across different rangeomorph taxa largely assume that branches underwent subdivision from a distal growth zone (Brasier and Antcliffe, 2009; Hoyal Cuthill and Conway Morris, 2014) (Table 2.1), and compare growth strategies across the rangeomorphs by considering inflationary growth and the appearance of new branches. In many uniterminal forms, growth appears to have proceeded in a similar way to that inferred in *Charnia* (e.g. *Trepassia wardae*; Laflamme *et al.*, 2007), but with some variation in the total number of first order branches, for example the imposition of an upper limit to the number of first order branches in certain taxa (Laflamme *et al*. 2012; Liu, Matthews and McIlroy, 2016).

In contrast to *Charnia*, *Fractofusus* (Fig. 2.1B) does not exhibit a clear linear relationship between the size of the organism and the number (and length) of first order branches (Gehling and Narbonne, 2007). In both described species of *Fractofusus*, first order branch bundles decrease in size distally in both directions along the growth axis, implying the presence of two distal growth tips (i.e. a bipolar growth axis) if it is assumed that the smallest branches are also the youngest (Seilacher, 1989; Brasier *et al.* 2012). *Fractofusus misrai*

exhibits additional variance, with asymmetric 'subsidiary' branches emerging from between first order branches (Gehling and Narbonne, 2007).

*Bradgatia* sp. (Fig. 2.1D, F) from Newfoundland, Canada, is the best-studied multiterminal rangeomorph, with four known morphotypes, each considered to represent a different ontogenetic stage (fig. 3.4 in Flude and Narbonne, 2008). First order branch lengths vary within populations from ~2 to 14 cm (fig. 8c in Flude and Narbonne, 2008; Liu *et al.* 2016), but do not appear to be tightly correlated with the morphotype-based ontogenetic sequence proposed for the taxon (Flude and Narbonne, 2008). More branches are visible in 'older' morphotypes of *Bradgatia* (the average number increasing from four to seven across the morphs; table 1 in Flude and Narbonne, 2008). However, it may be that the more diffuse form of the larger morphotypes means that more branches are visible, rather than that new branches were 'inserted' later in life (Flude and Narbonne, 2008). Within a single first order branch, the number of second order branches does not increase with branch length, varying between five and ten in most cases (Flude and Narbonne, 2008). Two hypotheses attempt to explain how the different orders of rangeomorph branches may have grown: (1) fractal growth, whereby one branch order reaches a critical size, triggering the development of the next, lower, order; and (2) a true inflationary model, where all branch orders are always present and grow in concert (Flude and Narbonne, 2008). *Bradgatia* is the only rangeomorph interpreted to possess secondary growth tips, added non-deterministically at the apex of large first order branches (Brasier and Antcliffe, 2009). *Hylaeculullus fordii* (Kenchington, Dunn and Wilby 2018), a multiterminal rangeomorph known from Charnwood Forest, exhibits a growth phenomenon termed 'eccentric branching' whereby a higher order (i.e. smaller) branch can occasionally revert to the morphology of its parent branch (i.e. one order lower). This phenomenon is only noted in up to the second branching order, and may indicate true biological modularity in these organisms as individual branches appear to show developmental independence (Kenchington *et al.,* 2018). Eccentric branching is interpreted as a damage response, given its variable presence or absence in specimens.

In summary, rangeomorphs have been considered to grow by one of two growth models: (1) the 'insertion' of new units and their subsequent inflation; or (2) the inflation of new units without additional 'insertion' (Table 1; Gehling and Narbonne, 2007; Bamforth, Narbonne and

Anderson, 2008; Flude and Narbonne, 2008). *Charnia*, *Fractofusus* and *Bradgatia* all exhibit smaller first order branches in smaller specimens, and *Charnia* shows an increase in the number of first order branches over time (although such a relationship is not seen in known ontogenetic stages of all rangeomorph taxa). All rangeomorphs for which ontogeny has been considered are interpreted to have grown *via* emergence of branches either from distally located generative zones positioned at the ends of a single, central proximodistal axis (as seen in the uniterminal and biterminal rangeomorphs), or through a central axis and the production of lateral, secondary growth tips (i.e. *Bradgatia*). Although the different ontogenetic patterns described in rangeomorphs can show divergence from the pattern seen in *Charnia*, I find no developmental evidence that would preclude their inclusion within a single clade.

### 2.3.2 Dickinsoniomorpha

Dickinsoniomorpha (Fig. 2.4) are defined as serially repetitive organisms with anterioposterior differentiation (Erwin *et al.* 2011 SOM), and include the genera *Dickinsonia*, *Yorgia*, *Windermeria* and *Andiva* (Erwin *et al.*, 2011). However, there is divergence of opinion concerning the composition of this morphogroup, and alternative groupings have been proposed, some of which include taxa such as *Spriggina* (Dzik and Ivantsov, 1999; Grazhdankin, 2014). Dickinsoniomorph taxa are all restricted to broadly shallow-marine settings ~559–551 Ma (Waggoner, 2003; Boag *et al.*, 2016).

Unlike the seemingly sessile rangeomorphs, dickinsoniomorphs, specifically *Dickinsonia* and *Yorgia waggoneri*, can be associated with impressions interpreted as trace fossils, suggesting a capacity for active locomotion (Ivantsov and Malakhovskaya, 2002; Gehling *et al.*, 2005; Sperling and Vinther, 2010; although see McIlroy, Brasier and Lang, 2009). Dickinsoniomorphs have been interpreted to exhibit evidence for internal anatomy, including gonads and diverticulae (e.g. Jenkins, 1992; Dzik, 2003), but such features have alternatively been interpreted as taphonomic artefacts (e.g. Brasier and Antcliffe, 2008). Biomarker data from specimens of *Dickinsonia* and *Andiva* from the White Sea suggest a steroid composition that is consistent with a holozoan affinity (Bobrobskiy *et al.* 2018).

Constructional units in dickinsoniomorphs have been likened to metazoan segments (Wade, 1972), but more recent interpretations have argued that they may represent only external annulations (Sperling and Vinther, 2010), features invoked by some authors as the precursor-state to a fully metameric bauplan (Chipman, 2010). Morphogenesis has been considered most commonly in *Dickinsonia costata* (e.g. Runnegar, 1982), a taxon that has been discussed in debates surrounding the evolution of bilaterality (Malakhov, 2004; Gold *et al.*, 2015).

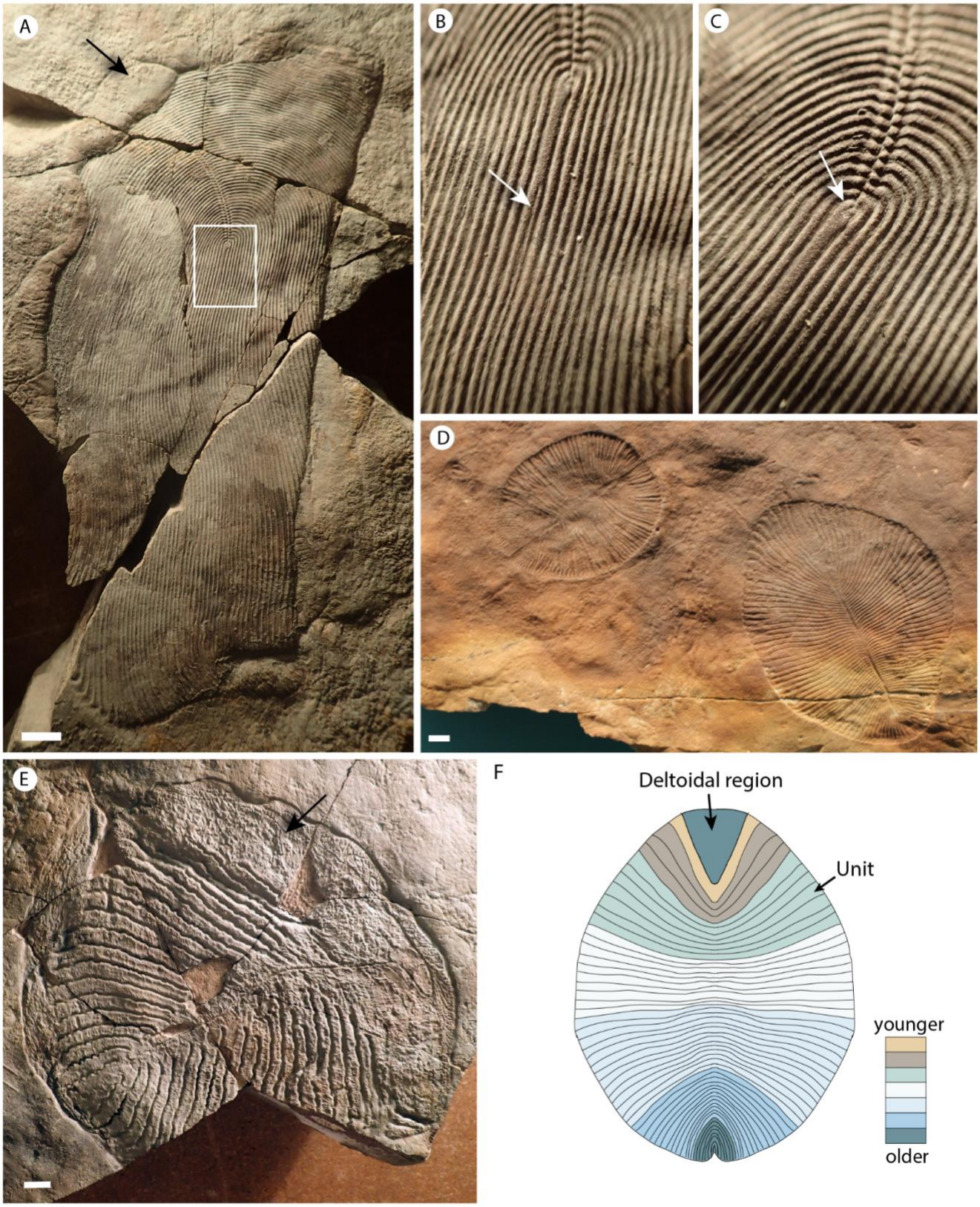
A
B
C
D
E
F
Deltoidal region
Unit
younger
older

**Figure 2.4:** Ediacaran dickinsoniomorph taxa. **A)** *Andiva ivantsovi,* White Sea, Russia. [Palaeontological Institute Moscow (PIN) specimen number 3993–5623]. (**B**, **C**) Enlargements of the boxed area in **A**. The areas of unit differentiation are indicated by white arrows, and undivided regions on *Andiva* and *Yorgia* are indicated by black arrows. **D)** *Dickinsonia costata,* South Australia [South Australia Museum (SAM) specimen numbers P49354 and P49355]. **E)** *Yorgia waggoneri,* White Sea, Russia (Holotype PIN 3993–5024). **F)** Stylised interpretation of growth in *Dickinsonia costata*, following the growth model proposed in Hoekzema *et al.* (2017). Scale bars = 10mm.

*(a) Dickinsonia*

*Dickinsonia costata* (Fig. 2.4D) is described from shallow-marine siliciclastic facies in South Australia and Russia. It exhibits an approximately oval outline, with distally expanding longitudinal units emanating from a visible central midline. Units are continuous across the midline (Runnegar, 1982; Gold *et al.*, 2015), imparting a bilateral symmetry. *D. costata* in Australia range from ~6–250 mm in length (Reid *et al.*, 2017), with size variants commonly considered to represent different ontogenetic stages (e.g. Evans *et al*, 2017; Hoekzema *et al*., 2017). Smaller specimens possess fewer units (as few as 12) than larger ones (which can have as many as 74; Sperling and Vinther, 2010). A triangular, undivided region seen in small specimens encompasses a proportionally smaller area of the body in increasingly larger specimens (the deltoidal region, e.g. Hoekzema *et al*., 2017), suggesting that in very early ontogenetic stages there may not have been any units at all (Ivantsov, 2007). The largest units are located close to the middle of the organism, not at either pole (Sperling and Vinther, 2010; Hoekzema *et al*., 2017). The position of the smallest units has often been used to infer the position of a growth zone (Runnegar, 1982; Ivantsov, 2007; Evans *et al.*, 2017), which has been described as being in a 'posterior' position (Ivantsov, 2007) with modules added terminally (Gold *et al.*, 2015; Evans *et al.*, 2017). Gold *et al.* (2015) follow Jacobs *et al.* (2005) in their definition of 'terminal addition', but figure a truly terminal generative zone (fig. 2 in Gold *et al.*, 2015). Evans *et al.* (2017) do not define 'terminal addition', but reference Gold *et al.* (2015) and also follow the definition of 'terminal addition' in Jacobs *et al.* (2005). However, recent work suggests that *Dickinsonia* instead added units at the opposing pole (Hoekzema *et al.*, 2017) *(Evans pers. comms.)*. The latter authors characterise growth of units within

populations of organisms interpreted to represent multiple ontogenetic stages, and present evidence for differentiation of new units from the margins of the deltoidal region itself. In this scenario, supported here, the generative zone of *Dickinsonia* may be considered pre-terminal (Fig. 2.4F). Further recent work has considered *Dickinsonia costata* to represent a paedomorphic variant of *Dickinsonia tenuis* (which possesses a greater unit count than *D. costata*; Zakrevskaya and Ivantsov, 2017).

These observations together suggest that *Dickinsonia* grew by the 'insertion' of new units, which then underwent subsequent inflation (see Runnegar, 1982; Fig. 2.4F). Larger specimens possess proportionally fewer units relative to their length, implying a reduction in the rate of unit addition (Evans *et al.*, 2017; Hoekzema *et al.*, 2017). However, there is variation in the number of units per specimen that is seemingly independent of (active?) contraction noted in many individuals (Evans *et al.*, 2017). *Dickinsonia* has been conflictingly interpreted to show both a pre-determined (Runnegar, 1982; Ivantsov, 2007) and an indeterminate (Retallack, 2007) mode of growth, but the apparent absence of size outliers belonging to *D. costata* appears to suggest that deterministic growth is more likely. The species *Dickinsonia rex*, however, could reach much greater sizes (~43 cm; Jenkins, 1992), suggesting that a determinate pattern of growth cannot yet be assumed for all *Dickinsonia* species.

*(b) Ontogenetic trends across dickinsoniomorphs*

Unlike *Dickinsonia*, *Andiva ivantsovi* (Fig. 2.4A–C) is not bilaterally symmetrical, bearing a glide plane of symmetry along its axial midline. *Andiva* does possess an undivided region, but whereas in *Dickinsonia* this region appears to diminish in size as the organism grew, its proportions relative to the overall organism are seemingly maintained in *Andiva* (Fedonkin, 2002). *Andiva* differs from *Dickinsonia* in several other regards. For example, there is seemingly no clear relationship between specimen size and number of units (see also Evans *et al.* 2018, fig 6b). Like *Andiva*, *Yorgia waggoneri* (Fig. 2.4E) also appears to possess an undivided region at all known stages of growth (Dzik and Ivantsov, 1999; Ivantsov, 2007). The smallest *Yorgia* specimens possess 10–12 independent units, while larger specimens can have up to 70 (i.e. 35 'isomer pairs'; Ivantsov and Fedonkin, 2001) aligned along a glide plane of symmetry. If *Dickinsonia*, *Andiva* and *Yorgia* are closely related, it is assumed they would possess a similarly positioned generative zone. I find potential evidence that *Andiva*

differentiated units from the opposite end to its undifferentiated area (i.e. its anti-deltoidal pole, see Hoekzema *et al*., 2017), based on the recognition of an apparently partially differentiated unit (Fig. 2.4A–C). While this could be alternatively interpreted as two overlying units, if correct this observation suggests that in *Andiva*, differentiation occurred at a truly terminal generative zone, at the opposite end to the undifferentiated region of the organism. Further work on a greater number of specimens is required, but it seems that the morphological differences previously outlined between *Dickinsonia* (bilaterally symmetrical with a proportionally variable deltoidal area) and *Andiva* (glide symmetry, and an undifferentiated crescentic region of fixed size relative to the body) may be corroborated by developmental differences, with growth progressing at different ends of the organisms with respect to their undifferentiated regions. Whether the undifferentiated deltoidal region of *Dickinsonia* and the crescentic region of *Andiva* are homologous remains to be determined. Our developmental comparisons do, however, raise the possibility that while *Dickinsonia* is arguably of the same morphological grade as other 'dickinsoniomorph' taxa, it may not ultimately belong to the same clade.

### 2.3.3 Erniettomorpha

Erniettomorphs (Fig. 2.5) are defined as serially repetitive organisms constructed entirely of tubular units arranged into fronds, 'sac-like' or 'canoe-like' benthic recliners, or flat-lying mats (SOM of Erwin *et al.*, 2011); this definition clearly encompasses a broad range of morphologies. Erniettomorphs are prominent constituents of the latest Ediacaran macrofossil assemblages of Namibia (~550–542 Ma) (Boag *et al.*, 2016; Darroch *et al.*, 2015), and Nevada (Smith *et al.*, 2017), yet their biology is little understood. Only two taxa, *Ernietta plateauensis* (a sac-like form) and *Pteridinium simplex* (a canoe-like form), have undergone detailed study (Elliott *et al.*, 2011, 2016; Ivantsov *et al.*, 2016). *Pteridinium simplex* is the most widely studied erniettomorph from an ontogenetic perspective, but whether its growth strategy is broadly applicable to all erniettomorphs is debatable given the morphological disparity of this group.

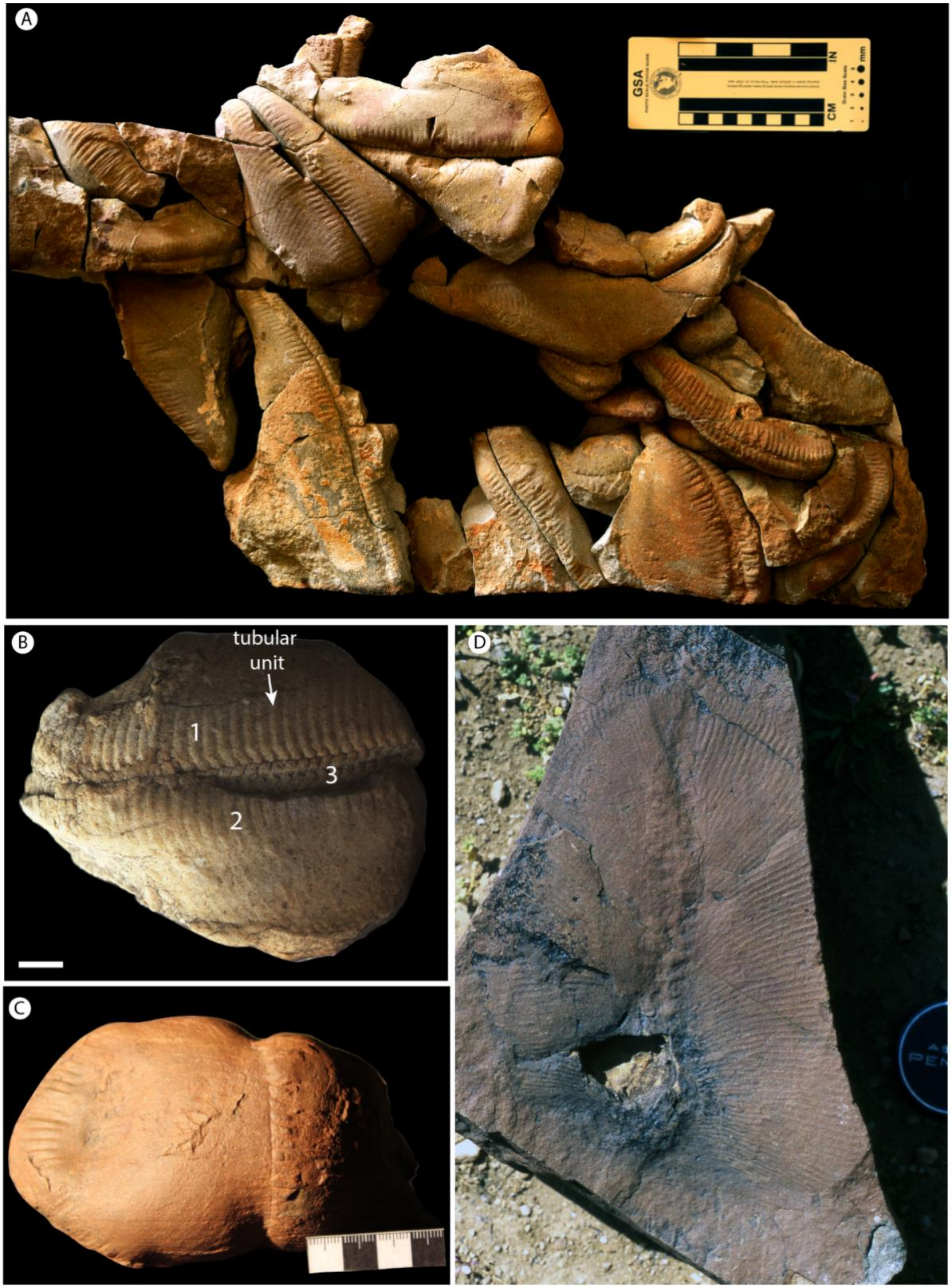


**Figure 2.5:** Ediacaran erniettomorph taxa. (**A**, **B**) *Pteridinium simplex,* Namibia. Numbers identifying the three identified vanes. **C)** *Swartpuntia germsii*, Namibia. **D)** *Ernietta plataensis*, Namibia. Scale bars = 10mm. Images courtesy of D. Grazhdankin (**A** and **B** from Grazhdankin and Seilacher, 2002), M.D Brasier (**C**), and M. Laflamme (**D**).

*(a) Pteridinium simplex*

*Pteridinium simplex* (Fig. 2.5A, B) appears to have been constructed of three vanes of tubular units (Fig. 2.5B) that meet in an alternating fashion at a central 'seam', imparting a glide plane of symmetry (Grazhdankin and Seilacher, 2002; Meyer *et al.*, 2014). Complete specimens range from 6.0 cm in length (along the central seam, displaying 26 units) to 19.2 cm (with 55 units) (Grazhdankin and Seilacher, 2002). The number and length (long axis) of individual units appears to correlate linearly with the organism's total length, but the height of the organism (the distance between the central seam and the termination of the long axis of the units) does not follow a similar relationship (Grazhdankin and Seilacher, 2002). The relationship between unit length and overall length reveals two distinct morphological groupings of *Pteridinium*; one showing a positive correlation between the two variables, and one showing no correlation (Grazhdankin and Seilacher, 2002). This ontogenetic variation may imply the presence of two distinct *Pteridinium* species, or may alternatively hint at ecophenotypic variation within the taxon (the study of which amongst the Ediacaran Macrobiota remains in its infancy: Kenchington and Wilby, 2017; Hoyal Cuthill and Conway Morris, 2017).

Specimens of *Pteridinium* can taper at one or both ends, with the tapering tip previously inferred to be the growth tip (Grazhdankin and Seilacher, 2002; Laflamme, Xiao and Kowalewski, 2009). *Pteridinium* has thus been variously considered as both unipolar (Grazhdankin and Seilacher, 2002) and bipolar (Laflamme *et al.*, 2009), although the lack of a tapering tip in some specimens may be a taphonomic bias (Seilacher, 1989). The distal-most unit can be positioned on either side of the central seam, suggesting that *Pteridinium* added units sequentially across its different vanes (Tojo *et al.*, 2007; although see Laflamme *et al.*, 2009). *Pteridinium* has previously been considered to grow mainly by the 'insertion' of new units over time (Laflamme *et al.*, 2009), but it appears that one morph also grew by the observable inflation of pre-existing units (Grazhdankin and Seilacher, 2002). Specimens that are ~6 cm long have been inferred to be immature (Grazhdankin and Seilacher, 2002), but there are no documented specimens of comparable size to those of the smallest rangeomorphs and dickinsoniomorphs (i.e. 10 mm or less).

*(b) Ontogenetic trends across the erniettomorphs*

The only other erniettomorph for which there is sufficient data to deduce ontogenetic information is *Ernietta plateauensis* (Fig. 2.5C). Unlike *Pteridinium*, the number of units remains relatively constant (23–28 on either side of the organism) across specimens of 35–55 mm in basal width (known size range 30–80 mm in width; Bouougri *et al.*, 2011). This suggests that growth took place primarily by the inflation of units, rather than by their continued insertion, at least in larger specimens (Ivantsov *et al.*, 2016). However, there has been considerable debate as to what constitutes a 'young' *Ernietta* (Hahn and Pflug, 1985; Runnegar, 1992; Schopf and Klein, 1992; Elliott *et al.*, 2016), and so I refrain from presenting an ontogenetic analysis of this taxon. Other erniettomorph taxa, such as *Swartpuntia* (Fig. 2.5D) (Narbonne, Saylor and Grotzinger, 1997), have received relatively little attention in terms of their morphogenesis. Before the morphogenesis of erniettomorphs can be reliably assessed, a re-evaluation of what constitutes membership of individual taxa is required. Consequently, it is currently not possible to compare ontogenetic processes between the erniettomorphs, and thus evaluate the biological reality of this morphogroup from a developmental perspective.

## 2.4 Developmental comparisons and phylogenetic inference

### 2.4.1 Extant taxa

Among the eukaryotes, serial repetitive growth is known in the chlorophyte, streptophyte, rhodophyte, and phaeophyte algae, land plants, fungi, and members of the Metazoa (Gold *et al.*, 2015). However, the processes by which these groups attain their essentially similar morphologies are very different. Plants and algae (red, green and brown) possess apical meristems, with the repeated re-specification of lateral organs along their length (Kuhlemeier, 2007). Each lateral organ displays developmental independence and, as such, these groups are classified as modular, displaying parallel modular growth, which results in an indeterminate morphology (Kaandorp, 2012; Fig. 2.6B). Brown algae, unlike plants and other algal groups that possess only one axial growth zone (Fig. 2.6C), can possess multiple axial growth zones located more basally (intercalary meristems: Charrier, le Bail and de

Reviers, 2012; Fig. 2.6D). Brown algal intercalary meristems have been interpreted as derived, whereas the apical meristem is considered plesiomorphic (Charrier *et al.*, 2012).

Fungi are also modular and grow from the tips of hyphae (Brand and Gow, 2009), but unlike the plants and the algae they lack a truly organismal body axis. Hyphae come together to form a fruiting body, rather than modules developing from a central structure as in plants. Moreover, fungi do not exhibit differentiation of new units over time. The fruiting body emerges following the formation of a 'hyphal knot' by multiply-branched hyphae, and subsequently differentiates into the constituent parts (e.g. in the button mushroom *Agaricus bisporus*; Umar and Van Griensven, 1997).

While not serially repetitive, since a lichen affinity has been advanced for some members of the Ediacaran Macrobiota (Retallack, 1994), their morphogenesis must be considered. Lichens are known to exhibit an indeterminate form, and so display parallel modular growth (e.g. fig. 1 in Suetina and Glotov, 2010).

Serial repetition is achieved in plants and algae by the presence of a totipotent meristem (a zone of cell proliferation that gives rise to the organs and tissues of a plant), but in colonial animals it can be achieved in a number of different ways. Within Cnidaria, coloniality is widespread, being most prevalent in the anthozoans and the hydrozoans, and with two main mechanisms of colonial growth at play. Monopodial growth is much like the meristematic growth seen in plants, whereby growth proceeds primarily from an (sub)apical growth tip; in athectate hydrozoans, lateral branches are specified successively and these then display monopodial growth themselves. In thectate hydroids, this same pattern of monopodial growth cannot occur due to the presence of the theca. In these forms, the apical stem tip acts in a fashion similar to a meristem, specifying new lateral shoots on both sides of the organism simultaneously (Berking, 2006). Sympodial growth involves the cessation of growth at the apical growth tip, and the re-specification of the 'apex' as outgrowths from successive lateral growth tips (Berking, 2006). Both monopodial and sympodial growth can occur either separately or concurrently. Some colonial anthozoans do not exhibit classical monopodial growth, with new branches emerging from a basal and pre-terminal growth zone in Pennatulacea (Antcliffe and Brasier, 2007). Colonial cnidarians are also known to show colony

polymorphism (discontinuous variation in zooid morphology within colonies: Hyman, 1940*a*; Boardman, Cheetham and Oliver, 1973). In such cnidarians, repeated units tend to appear in sets, or whorls (Gold *et al.*, 2015).

Extant members of Porifera do not show a serially repetitive body plan in the same way as certain cnidarians, and do not display the same level of colonial integration (i.e. the division of labour). However, certain sponges (e.g. the demosponge *Callyspongia vaginalis*) are constructed of serially repeated units. Recent work has elucidated a broad repertoire of developmental regulatory genes in the Porifera, suggesting there is no *de facto* reason that early sponges couldn't have exhibited greater morphological complexity (Leininger *et al.*, 2014). The phylogenetic position of the Placozoa remains in flux (Collins, 1998; Cannon *et al.*, 2016; Laumer *et al.* 2018), and there is not enough information to reconstruct their ancestral state (the same is true for Ctenophora), and the presumably simplified morphology of extant placozoans, and the derived nature of extant ctenophores, means I cannot exclude either group from the Ediacaran debate.

Many colonial bilaterians (belonging to Rouphozoa and Gnathifera; Laumer *et al.*, 2015) tend to show, in the broadest sense, a more diffuse form of colonial growth (Fig. 2.6E). In bryozoans, which can possess frondose or arborescent forms, new zooids emerge by budding, with the pattern of budding being almost species specific and determining the form of the colony (Hyman, 1940*b*). The entoprocts, once considered to be members of Bryozoa, are largely colonial in form. Entoprocts often grow through laterally spreading stolons, with vertically projecting zooids emerging at intervals; they do not form arborescent colonies. Meanwhile the rotifers display an aggregative form of colonialism, whereby juveniles become tangled up and eventually adhere to each other by production of an adhesive string from a foot gland (Surface, 1906).

The serially repetitive structures observed in members of the segmented unitary Bilateria – the arthropods, annelids and chordates – develop largely through the process of posterior growth *via* the specification of units in parallel with the elongation of the anterior–posterior axis (Jacobs *et al.*, 2005). Whereas in many serially repetitive organisms there is a disjunct between the growth of individual units and the growth of the main body axis, the two are

concurrent in the segmented Bilateria. The specification of units is sequential in most of these bilaterians, but there are exceptions, such as the long-germ-band insects (e.g. *Drosophila melanogaster*), which specify the entire anterior–posterior axis simultaneously (Liu and Kaufman, 2005). The patterns imparted by different forms of segmentation can manifest in different ways. Organisms can be homonomously segmented, whereby segments are largely identical, or groups of segments performing similar tasks may group together into functional units known as tagmata.

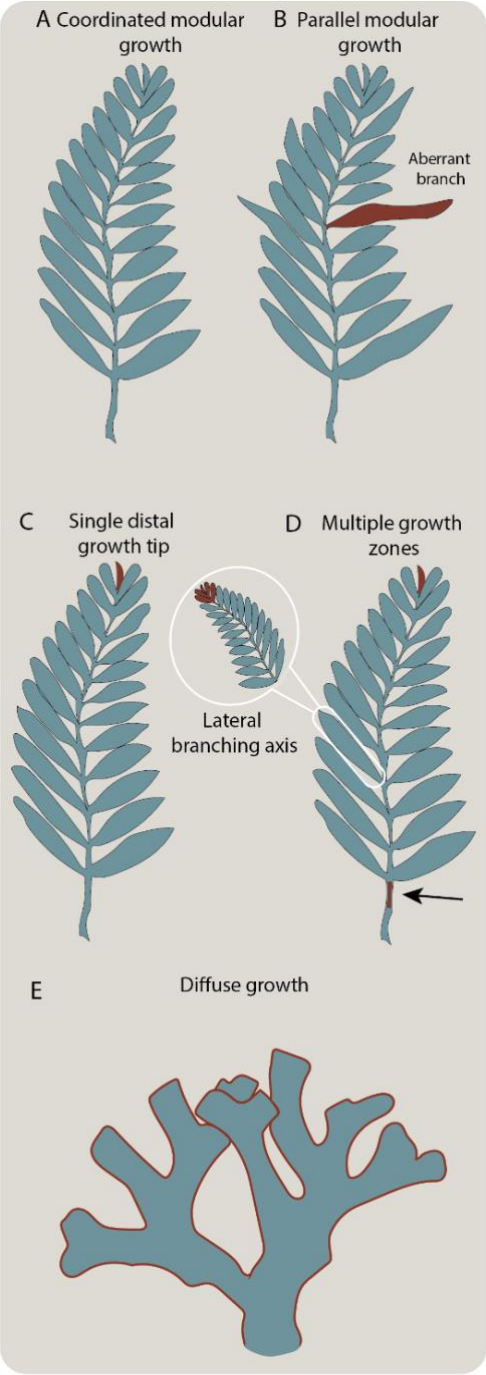


**Figure 2.6:** Schematic diagram showing the forms of growth observed in extant clades with serial repetition of component units; red indicates the style/feature of growth discussed. **A)** Coordinated modular growth, seen in certain metazoan groups. **B)** Parallel modular growth, common in plants and red, green and brown algae, with an aberrant branch highlighted in red. (**C**, **D**) Positioning of different central (additional growth zone highlighted with black arrow) and lateral growth zones/tips in extant serially repetitive groups. Single apical axes are seen in green and red algal groups, whereas multiple axes are seen in various metazoan and brown-algal groups. **E)** Diffuse growth, as seen in colonial bilaterian groups characterised by colony-wide tip growth.

### 2.4.2 Implications for the Ediacaran Macrobiota

Proposed members of the rangeomorphs, dickinsoniomorphs and erniettomorphs have all been described as growing by either the differentiation of new units, the inflation of pre-existing units (at known ontogenetic stages), or a combination of the two (Table 2.1). Description of growth by the differentiation of new units and/or their subsequent expansion alone is, however, uninformative for constraining phylogenetic affinity, since this method of formulating new units is universal among multicellular eukaryotic groups (Bonner, 1952). The absence of data on the very earliest growth stages (of a few millimetres or less) in Ediacaran taxa also hampers efforts to determine the point at which differentiation occurred in the life cycle in some taxa.

The position of the generative zone is potentially a more useful developmental character, but identification of this trait in rangeomorphs, dickinsoniomorphs, and erniettomorphs remains difficult since the assumption that the position of the smallest units correlates with the position of the generative zone has recently been questioned (Hoekzema *et al*., 2017). In the following discussion, I assume that previously ascribed generative zones as discussed in the above sections are correct, but note that such assumptions remain unproven.

Rangeomorphs exhibit a non-deviant form (i.e. aberrant-length branches are not observed in thousands of studied specimens). A notable exception to this are certain multiterminal rangeomorphs, including the recently described *Hylaecullulus fordii* (Kenchington *et al.,* 2018), which displays eccentric branching. However, as this phenomenon is compellingly attributed to a damage response (overcompensatory branching), it is not interpreted as part of base ontogenetic pattern. It is, therefore, highly likely that rangeomorphs do not exhibit the parallel modular growth characteristic of non-metazoan serially repetitive groups. Their shape is seemingly constrained at both the organismal level, and at the level of individual branches (including subsidiary branches; Gehling and Narbonne, 2007), across the known ontogenetic series.

Unlike Fungi, rangeomorphs exhibit the differentiation of new units. The presence of a basal growth zone (in the stem and potentially in some of the lowermost first order branches), as well as an apical one, at least in *Charnia*, would ally them further with Eumetazoa (but of course our understanding of plesiomorphic states in early diverging metazoans is preliminary while the phylogenetic positions of non-bilaterian phyla remain in flux [Whelan *et al.* 2013; Simion *et al.* 2017; Laumer *et al.* 2018). The presence of discrete (as opposed to diffuse) growth tips would argue against affinities with most members of Rouphozoa and Gnathifera, but the likely presence of multiple axial growth zones (in *Charnia*) and potential secondary growth tips (in *Bradgatia*), is reconcilable with known variation in members of the colonial cnidarians. Based on current data, I cannot rule out a stem-metazoan affinity for rangeomorphs (if the Porifera are the sister lineage to all other metazoans; Pisani *et al.*, 2015), or, indeed, a stem-poriferan affinity, but the general paucity, as opposed to conflict, of data prevents further assessment (Fig. 2.7). I do not consider a ctenophore affinity likely since both

extant ctenophores and organisms considered to be stem-group ctenophores, including the Ediacaran *Eoandromeda*, are considered to be motile (Tang *et al.*, 2011).

Dickinsoniomorphs as currently defined also lack evidence of parallel modularity, and show the differentiation of new units across ontogeny, precluding algal and fungal phylogenetic affinities. When combined with trace fossil evidence for motility, and anatomical evidence (Sperling and Vinther, 2010), this developmental constraint likely indicates that they are metazoan. The data of Hoekzema *et al*. (2017) suggest that *Dickinsonia* may have possessed a pre-terminal growth zone along with concurrent inflative growth in lateral units and the main growth axis, which can be reconciled with the basal and pre-terminal generative zone of extant segmented bilaterians (Fig. 2.7A). There are, of course, exceptions to this rule, such as Onychophora (which grow from a true terminus; Anderson, 1973), or Nematoida (where a secondary loss of serially repetitive units makes confirmation of a pre-terminal growth zone difficult), but these conditions have been considered to be derived from an ancestral pattern of pre-terminal addition (Jacobs *et al.*, 2005). The monopodial serially repetitive cnidarians also show a pre-terminal mode of extension rather than a true terminal growth zone, so a pre-terminal generative zone for *Dickinsonia* remains compatible with such organisms. However, organisms of cnidarian grade may also exhibit truly terminal differentiation (e.g. monopodially growing athectate hydrozoans; Berking, 2006). A placozoan affinity for *Dickinsonia* (Sperling and Vinther, 2010) is difficult to evaluate on developmental grounds given the low diversity and disparity of extant placozoans, and remains a viable possibility (Fig. 2.7). The potential for a truly terminal growth zone in *Andiva* (Fig. 2.7) could suggest that a non-bilaterian affinity is possible for at least some dickinsoniomorph taxa.

Currently, the erniettomorphs are too poorly understood to infer their phylogenetic position from developmental data. Members of Erniettomorpha have been considered to show morphological similarities to members of the annulated *Dickinsonia*-like taxa (e.g. Budd and Jensen, 2017), but whether this evidences a close phylogenetic relationship is unclear. The relative consistency of overall form in erniettomorphs suggests that they do not exhibit parallel modular growth and, thus, they are unlikely to be plants or algae. Continuous differentiation of new units in *Pteridinium* seemingly rules out a fungal affinity. There are no current data to exclude *Pteridinium* from Metazoa, but there is similarly no additional

evidence to support a metazoan affinity. Given our poor knowledge of erniettomorphs, I cannot currently extrapolate from *Pteridinium* to other organisms. Indeed, this review has highlighted significant gaps in knowledge of development in multiple Ediacaran taxa, as well as taxonomic issues that require resolution before morphogenesis can be meaningfully addressed in other morphogroups.

## 2.5 Implications for developmental evolution

Developmental evidence supports a metazoan affinity for rangeomorphs (Fig. 2.7B). Their multiple axial growth zones, as well as their asymmetric glide plane of symmetry, apparent in all known life stages, argue against most bilaterian affiliations, but I note that forms of glide symmetry are known in bilaterian taxa including echinoids (e.g. between plates in the interambulacral zone) and graptolites (e.g. *Eoglyptograptus*). There are also rare reports of bilateral symmetry at higher branching orders in some rangeomorphs (figs 3D, 4A, 5C in Flude and Narbonne, 2008), potentially revealing additional complexity in the axial patterning of these organisms, and illustrating that symmetry may not represent a reliable phylogenetic indicator for Ediacaran taxa.

The rangeomorphs appear to have one main body axis and one lateral branching axis, an arrangement very similar to various cnidarian organisms (Wantabe *et al.*, 2014), with which they also share developmental similarities, namely a conserved form and potential positioning of the generative zone. The possibility that rangeomorphs possessed a third body axis (akin to the dorso-ventral axis), cannot yet be excluded, but seems unlikely given evidence to suggest that some rangeomorphs were identical on both 'sides' (e.g. fig. 3 in Seilacher, 1992; fig. 5.2 in Wilby *et al*., 2015; although see Gehling and Narbonne, 2007, for a discussion of taphonomic reasons for why a third vane may not be preserved in *Fractofusus*). Sponges are conventionally interpreted to possess just one principal body axis, but a reduction in the number of body axes may have occurred as part of any secondary simplification (e.g. Ferrier *et al.* 2015) within crown groups of early branching metazoans. Therefore, resolution of the rangeomorphs as falling within the metazoan stem or, indeed, Porifera, cannot be excluded.

The rangeomorphs do not show either true radial symmetry or bilateral symmetry, but the possibility that rangeomorphs like *Charnia* displayed biradial symmetry could prove informative. If the rangeomorphs belong to the eumetazoan stem group, their possible possession of biradial symmetry could support the notion that biradiality was a precursor to bilateral symmetry in metazoans (Martindale and Henry, 1998). This is particularly pertinent given that the rangeomorphs may themselves have possessed bilateral symmetry at smaller branch orders (Flude and Narbonne, 2008). Alternatively, tentative biradial symmetry could support the idea that early metazoans experimented with variants of radial symmetry independent of phylogeny (see also the putative stem-ctenophore *Eoandromeda* which exhibits octoradial symmetry, the triradial form *Tribachidium*, tetraradial *Conomedusites*, and pentaradial *Arkarua*; Xiao and Laflamme, 2009).

*Dickinsonia*, like rangeomorphs, appears to possess one major body axis and one lateral axis, with insufficient evidence to determine differentiation across a third axis [although see Evans *et al.* (2017) for discussion of *Dickinsonia* 'height']. I resolve *Dickinsonia* as a member of total-group Metazoa (Fig. 2.7B), likely within the Placozoa plus Eumetazoa total group, on the basis of the continued differentiation of units across the life-cycle and the absence of parallel modular growth, combined with the apparent capacity for active locomotion (see Hoekzema *et al*., 2017).

Consideration of *Eoandromeda octobrachiata* as a stem-ctenophore (Tang *et al.*, 2011) has resulted in attempts to find homology between the body axes of radial and non-radial Ediacaran taxa. The asymmetric head region of *Yorgia* has been speculatively likened to two of the three branch-like structures that make up *Tribrachidium* (Budd and Jensen, 2017), implying axial homology between the dorso-ventral axis of *Tribachidium* and the 'anteroposterior' axis of dickinsoniomorphs. In the absence of an asymmetric undivided region in some dickinsoniomorphs, and even in some *Dickinsonia* specimens, I do not consider that there are sufficient grounds to consider these axes to be homologous.

If members of the Dickinsoniomorpha can be resolved with bilaterians, they may prove informative on the appearance of bilaterian characters. In the evolution of metamerism, a determinate form (i.e. a pre-determined number of units) likely appeared late; well after the

initial appearance of true metamerism (Vroomans, Hogeweg and Tusscher, 2016). In *Dickinsonia*, organisms of different sizes display variable numbers of units, such that the number of units does not appear pre-determined (Evans *et al.*, 2017; Hoekzema *et al.*, 2017). Therefore, if *Dickinsonia* was truly metameric (and future work is required to establish this), the fossil data would appear to concur with these prior theoretical predictions. Interestingly, the positions of putative internal anatomical structures preserved within *Dickinsonia* (e.g. Dzik and Ivantsov, 2002; Zhang and Reitner, 2006) do not correlate with the positions of the visible units considered to be on the exterior of the organism. As such, if these structures represent true biological features, and these organisms were truly segmented, they must have been heteronomously so (i.e. where segments are non-identical), possessing tagmata. While it is likely that the three main segmented bilaterian groups all developed segmentation independently of each other, it appears that the homonomous state is plesiomorphic to the arthropods and annelids (being present in the stem-lineages of these clades if I discount highly derived tagma in the head regions; e.g. Parry, Vinther and Edgecombe, 2015; Ortega-Hernández, Janssen and Budd, 2016), whereas heteronomous segmentation appears plesiomorphic to the vertebrates [for example, in the vertebral column (Jacobs *et al.*, 2005)]. I therefore find that dickinsoniomorphs do not sit comfortably in the stem groups of the annelids or arthropods on account of their seemingly heteronomous state. However, the absence of any chordate diagnostic characters means they cannot be reconciled with chordates either. Therefore, if members of Dickinsoniomorpha are resolved as being segmented, in this scenario I consider it most likely that they represent a bilaterian group that independently adopted a segmented form.

Another consideration is that some dickinsoniomorphs (perhaps most notably *Yorgia*) possess glide symmetry, not bilateral symmetry, meaning that under the scenario in which the dickinsoniomorphs do represent a coherent clade, any ‘segments’ would be discontinuous across the midline. Two possibilities then arise: *Yorgia* is not segmented, but does possess external annulations that may or may not be a precursor state to true segmentation; or conversely, *Yorgia* does display a form of derived segmentation similar to that seen in long-germ-band insects today, where the ‘segments’ are not the fundamental unit. In these cases, parasegments cross segment boundaries (Martinez-Arias and Lawrence, 1985), and pattern the embryo of certain insects (e.g. *Drosophila*).

The resolution of these organisms as falling within Metazoa does not in itself help us to resolve between their potential body axes. It is broadly true that sponges have one main body axis, diploblasts have two and triploblasts have three, and that these main axes are patterned by the same pathways and gradients, and so may be homologous (e.g. Leininger *et al.*, 2014). Wingless-related integration site (Wnt) patterning across both the oral–aboral and anterior–posterior axes (e.g. Holstein, 2012) may suggest that the primary axis across Eumetazoa is homologous, and similar Wnt patterning across the primary body axis of sponges suggests that the primary body axis across all Metazoa may be homologous (Leininger *et al.*, 2014). Similarly, bone morphogenetic protein (BMP) signalling across the directive and dorso-ventral axes (Matus *et al.*, 2006; Genikhovich *et al*., 2015) may or may not suggest homology across Eumetazoa. However, many animal groups show major shifts in axial patterning, and so using morphology alone can lead to difficulty in identifying even analogous axes (e.g. the secondary acquisition of a pentameral body plan in starfish and sea urchins confounds identification of the anterior–posterior axis). Cnidarians, as a group, are almost typified by a number of excursions into radial symmetry (perhaps from a bilateral ancestor; Dzik, Balinski and Sun, 2017), making the directive axis hard to identify from morphology alone. There are also examples of organisation along the dorso-ventral axis being inverted between arthropods and vertebrates [i.e. the reversal of positioning of the nerve cord (e.g. Denes *et al.*, 2007)]. Many Ediacaran macro-organisms inferred to represent ancient animals are themselves characterised by excursions into forms of radial symmetry, potentially independent of phylogeny, making points of homology difficult to ascertain. If axis homology can be proven by resolution of phylogenetic placement, these fossils could be interpreted to represent a primitive diversity of body plans, perhaps suggesting that successive disruptions and alterations to the planes of these body axes may be plesiomorphic. However, these data also warn of the problems of inferring homology across the body axes of diploblasts and triploblasts; if *Dickinsonia* is resolved as being a placozoan, or cnidarian, then definition of its main body axis as anterior–posterior (e.g SOM of Erwin *et al.*, 2011) is inappropriate. Until axis homology can be identified, it seems prudent to use phylogenetically neutral terms in differentiating across body axes.

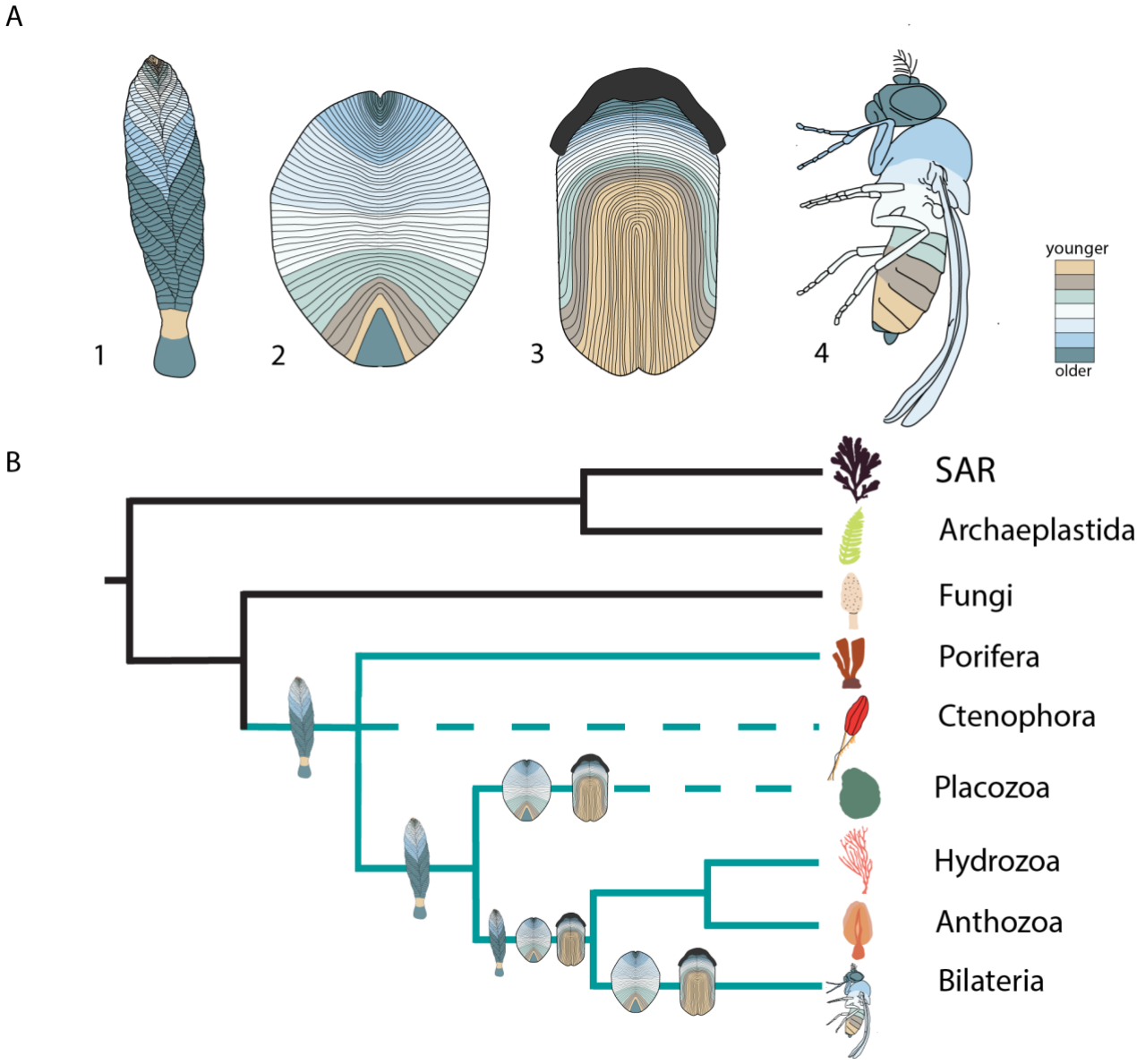


**Figure 2.7: A)** Interpretive growth models of 1, *Charnia masoni;* 2, *Dickinsonia costata;* 3. *Andiva ivantsovi;* 4. An extant bilaterian comparator. **B)** A simplified eukaryote phylogeny including only groups with serially repetitive body plans to which the Ediacaran morphogroups have been compared. SAR = Stramenopiles, Alveolates and Rhizaria. The suggested phylogenetic positions of *Charnia, Dickinsonia* and *Andiva* are presented as discussed in the text (I include *Andiva* as possibly being resolved within the Bialteria because although our morphological data may suggest a truly terminal generative zone, this is based on one specimen and additional data are required to confirm or refute this). Green represents metazoan lineages. Dashed lines indicate the possible position of a group (owing to uncertainty surrounding the phylogeny of the early-diverging Metazoa; e.g. Dunn *et al.* 2014).

## 2.6 Conclusions

There is significant potential to improve our knowledge of development in Ediacaran macro-organisms, but the synthesis of existing data allows us to refute several previously proposed phylogenetic affinities for key Ediacaran taxa. Analysis of development in rangeomorphs and dickinsoniomorphs reveals congruence with aspects of metazoan development.

I conclude that developmental data alone allow me to identify *Dickinsonia*, *Andiva*, and the rangeomorphs as early metazoans. Morphogenesis offers great promise for disentangling Ediacaran phylogenetic relationships and the evolution of development. Although the study of ontogeny is the study of change over time, by adopting a largely morphological approach when considering Ediacaran organisms, the 'change' has been largely overlooked. Future study of populations of organisms will allow better quantification of this change, as well as the production of growth models, both of which will ultimately increase the precision of phylogenetic resolution of Ediacaran organisms.

The recognition of some the most enigmatic members of Ediacaran fossil assemblages as probable metazoans offers support to recent suggestions of considerable developmental complexity in early-branching metazoans (e.g. Ferrier, 2015), and lends credence to the idea that the early metazoan tree cannot be rationalised in terms of gradually increasing complexity, but may have followed a much more cryptic path.

# Chapter 3:

## Anatomical and ontogenetic reassessment of the Ediacaran frond *Arborea arborea* and its placement within total group Eumetazoa:

**Author contributions:** The sections of this chapter based on Australian material are in press at a peer reviewed journal

**Dunn, F. S.,** Liu, A. G. and Gehling, J. G. Anatomical and ontogenetic reassessment of the Ediacaran frond *Arborea arborea* and its placement within total group Eumetazoa. *Palaeontology.*

The study on Australian material was designed by F.S.D and A.G.L, and both F.S.D and A.G.L contributed to data collection. F.S.D. conduced all analyses and produced the first draft of the manuscript, and F.S.D, A.G.L and James G. Gehling provided feedback on the manuscript. F.S.D is the lead author of this paper. The study on British material was designed by F.S.D and Philip R. Wilby. F.S.D. conducted all analyses and produced the first draft of the text. F.S.D and P.R.W provided comments on the text. F.S.D contributed ~85% of the work presented in this chapter.

## Abstract:

Organisms with a frondose body plan are amongst the oldest and most enigmatic members of the soft-bodied Ediacaran Macrobiota. Appraisal of specimens from the late Ediacaran Ediacara Member of South Australia reveals that the frondose taxon *Arborea arborea* possessed a fluid-filled holdfast disc, the size and form of which could vary within populations. Moldic preservation of internal anatomical features within the frond provides evidence for tissue differentiation, and for bundles of tubular structures within the stalk of the organism. These structures connect in a fascicled arrangement to individual lateral branches, before dividing further into individual units housed on those branches. The observed fascicled branching arrangement, which seemingly connects individual units to the main organism, is consistent with a biologically modular construction for *Arborea*, and raises the possibility of a colonial organisation. In conjunction with morphological characters previously recognised by other authors, including apical-basal and front-back differentiation, I propose that to the exclusion of all alternative known possibilities, *Arborea* was a total group eumetazoan.

Further, I describe specimens previously referred to as 'arboreomorph undet.' from Charnwood Forest, U.K. as *Arborea.* This description extends the known stratigraphic range of *Arborea* by between seven and twelve million years, and expands the known environmental tolerances of the taxon into deep-water palaeoenvironments.

## 3.1 Introduction:

The Flinders Ranges of South Australia (Fig. 3.1) offer an exceptional record of Ediacaran taxa within fine- to coarse-grained sandstones of the Ediacara Member of the Rawnsley Quartzite (Droser *et al.* 2017). This unit documents a variety of shallow-marine and deltaic depositional environments (Gehling 2000; Gehling and Droser 2013; Callow, Brasier and McIlroy 2013; Tarhan *et al.* 2017), and contains the impressions of thousands of organisms representing at least 30 distinct macrofossil taxa. Although the precise mechanism by which these fossils are preserved is a matter of considerable debate (Gehling 1999; Retallack 2007; Liu 2016; Tarhan *et al.* 2016, 2018; Liu *et al.* 2019), there is a general consensus that Ediacara Member palaeoenvironments were reasonably high-energy marine settings, and that the seafloor that the organisms inhabited was covered by benthic microbial mat communities (Gehling and Droser 2009; Droser *et al.* 2017; Tarhan *et al.* 2017).

Fossil assemblages of the Ediacara Member are perhaps most widely known for including some of the oldest candidate bilaterian animals (Gold *et al.* 2015; Cunningham *et al.* 2017), including *Kimberella* (Gehling, Runnegar and Droser 2014; Droser and Gehling 2015), *Parvancorina* (Paterson *et al.* 2017; Darroch *et al.* 2017; Coutts *et al.* 2017), and *Dickinsonia* (Evans *et al.* 2017; Hoekzema *et al.* 2017; Bobrovskiy *et al*. 2018; though see Sperling and Vinther 2010). Alongside these taxa, frondose organisms (Glaessner 1971) assigned to the unranked morphogroups Rangeomorpha and Arboreomorpha (Erwin *et al.* 2011) represent a comparatively little-studied component. Frondose taxa are better known from older, deep-marine Ediacaran palaeoenvironments in Newfoundland (Canada) and England (Liu, Kenchington and Mitchell 2015), but in the Ediacara Member they occur in shallow-marine delta front environments, preserved in sheet-flow and mass-flow deposits (Gehling and Droser 2013; see also Tarhan *et al.* 2016). Frondose taxa represented amongst the Ediacara Member include *Charnia* (Gehling and Droser 2013), *Bradgatia* (Droser and Gehling 2015), and *Pambikalbae* (Jenkins and Nedin 2007). However, numerous discoidal impressions, initially interpreted as medusoids (Glaessner 1984) but more recently reinterpreted as holdfast structures of frondose organisms (Tarhan *et al.* 2015), may indicate that frondose taxa were reasonably abundant within all Ediacara Member palaeoenvironments, although

taphonomic variation in disc expression currently precludes identification of original taxa in situations where the frond is absent (Gehling, Narbonne and Anderson, 2000; Burzynski and Narbonne 2015; Tarhan *et al.* 2015).

The most common frondose taxon in the Ediacara Member is *Arborea arborea* (Glaessner and Daily 1959), the organism after which the morphogroup Arboreomorpha is named (Laflamme and Narbonne 2008a; Erwin *et al.* 2011; Laflamme *et al.* 2018). *A. arborea* can be common on individual bedding surfaces within wave-base, sheet-flow and mass-flow facies (Laflamme *et al.* 2018; see *Charniodiscus* in Gehling and Droser 2013), and also occurs in low densities alongside more typical components of the Ediacaran biota in shoreface and delta-front facies (Coutts, Gehling and García-Bellido 2016). Some *Arborea* specimens may have exceeded lengths of three metres (Fig. 3.2), making this one of the largest known Ediacaran macro-organisms. A detailed reassessment of frondose taxa in South Australia synonymised specimens previously assigned to *Charniodiscus oppositus*, *Charniodiscus arboreus*, *Rangea arborea*, *Arborea arborea*, and even some *Charnia* sp. within *A. arborea*, following determination of the three-dimensional structure of *Arborea* branches (Laflamme *et al.* 2018). That study diagnosed *Arborea* as a bifoliate frond with second order branches that lack rangeomorph sub-divisions (consistent with Laflamme and Narbonne 2008; Erwin *et al.* 2011; Brasier, Antcliffe and Liu 2012; Laflamme *et al.* 2018): a bifoliate arrangement is supposedly distinct from that observed in the type *Charniodiscus* material from the UK. Whereas rangeomorph taxa have historically been assigned to multiple, often contradictory, phylogenetic positions within the eukaryotes (summarised in Chapter one), *Arborea* has only seriously been proposed to fall within either the hypothetical phyla Petalonamae (Pflug 1970; Pflug 1972; Hoyal Cuthill and Han 2018) and Vendobionta (formerly Kingdom Vendozoa, more recently considered a class or order of rhizoid protists; Seilacher 1989, 2007; Buss and Seilacher 1994; Seilacher *et al.,* 2003), or the Cnidaria (Jenkins and Gehling 1978). I reassess the morphology of multiple *Arborea* specimens from South Australia, and build upon recent studies (Laflamme *et al.* 2018) to propose a new model for *Arborea* anatomy.

## 3.2 Materials and methods:

I assessed 56 specimens that have either been historically assigned to *Arborea*, or recently synonymised within that taxon (Laflamme *et al.* 2018), in the collections of the South Australia Museum (SAM). A complete specimen list can be found in Appendix one. Specimens were collected from the Ediacara Member of the Rawnsley Quartzite between 1957 and 2015, within the Ediacara Conservation Park, the Flinders Ranges National Park, and National Heritage Site Nilpena (Fig. 3.1). Many of the studied specimens are incomplete, and when originally catalogued by their discoverers (who include M. Wade, M. Glaessner, W. Sun, R. Jenkins, and J. Gehling), were assigned to several different taxa. I follow recent synonymisation (Laflamme *et al.* 2018) of these specimens, and care has been taken to base the principal findings of this study only on specimens I am confident derive from a single taxon conforming to this most recent diagnosis of *Arborea arborea* (Laflamme *et al.* 2018).

Most of the studied specimens are preserved as positive hyporelief moldic impressions on the bases of sandstone beds, but some constitute composite hyporelief molds of original external and internal anatomy. A small number of specimens are preserved in full three dimensions as sand-filled casts typically documenting external morphology (Laflamme *et al.* 2018), while one new surface (from Nilpena, Fig. 3.2) possesses very large specimens preserved in positive epirelief. These latter specimens remain *in situ* in the field. Key anatomical findings of Laflamme *et al.* (2018) include evidence for 'dorso-ventral' differentiation in *Arborea*, the inferred preservation of internal structures, and the ability for sediment to become incorporated within the specimens. I confirm those findings, but interpret several additional anatomical features to be biologically informative. I refrain from using phylogenetically loaded terminology in our description of *Arborea*, for reasons discussed in Chapter two.

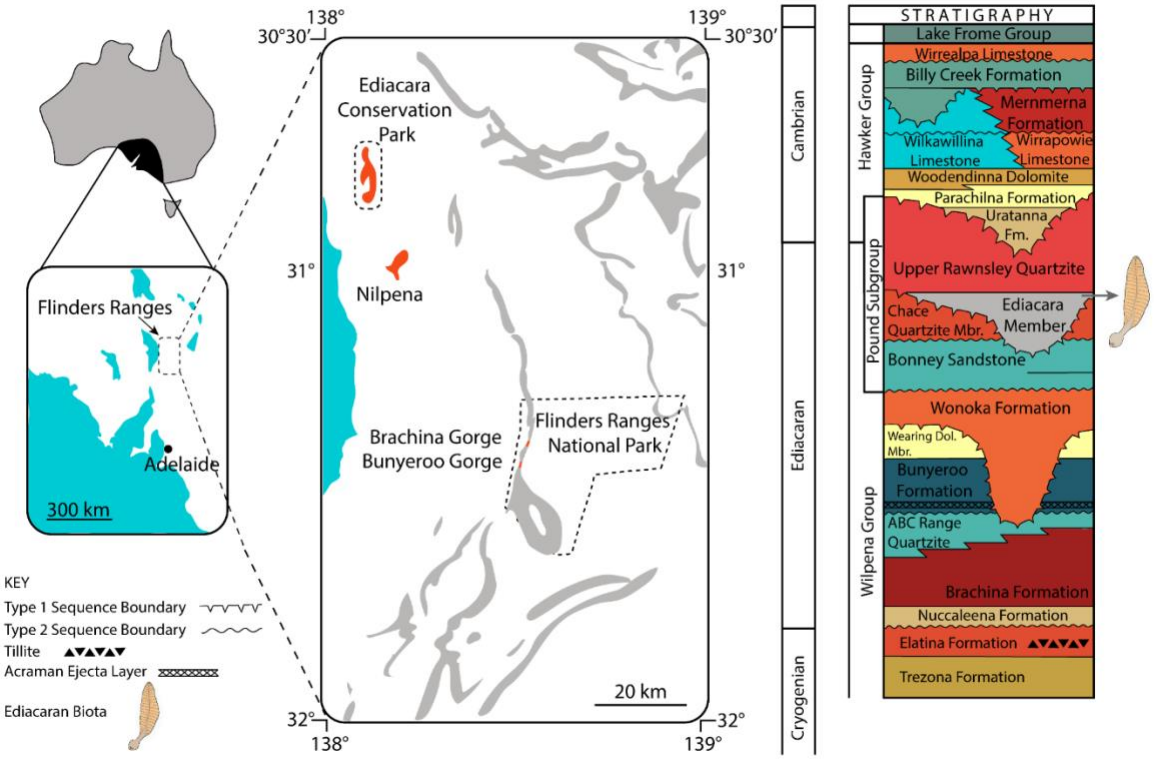


**Figure 3.1**. Maps showing the location of the Flinders Ranges and field collection sites (in red) within Australia, and the Cryogenian to Cambrian stratigraphy of the Flinders Ranges (after Gehling and Droser 2012). Fossils of the Ediacaran Macrobiota examined during this study lie within the Ediacara Member. In a type one sequence boundary, the shoreline experiences a fall in sea level, and so rivers will incise the shoreline and a forced regression will form. In a type two sequence there is no fall in sea level at the shoreline; a forced regression does not form.

## 3.3 Results:

*Arborea arborea* is composed of a holdfast, a stem, and an ovate, leaf-like frond comprising two rows of lateral branches (following Runnegar 1995) emanating from either side of a central stalk (Fig. 3.3A). Each branch within the frond comprises smaller sub-divisions (here called units, previously referred to as second order branches) that appear to lie behind a covering structure, or 'pod' (*sensu* Laflamme and Narbonne, 2008a; Fig. 3.4). Known *Arborea* specimens range in size from complete specimens of just a few centimetres in length to

incomplete fronds of over one metre, and are interpreted to have been approximately three metres in length when cmoplete (Fig 3.2). The smallest studied specimen (SAM P40785; Fig. 3.4A) possesses ~19 lateral branches per row and is 3.5 cm in total length, while specimens longer than ~4.5 cm in length (SAM P48727; Fig. 3.4E, or P19690a; Fig. 3.3A) possess >30 lateral branches. One large incomplete frond (>>74.45 cm) possesses at least 33 branches (SAM P40858), while a newly discovered specimen (incomplete at >>150 cm) has >49 (Fig. 3.2). The frond outline transitions from tapering (in terms of branch length) at both tips in smaller specimens (fusiform), to tapering primarily at the apical tip. In a specimen ~4.5 cm in length (Fig. 3.4E) the basal-most branches are ~40% of the length of the longest branches, whereas in a specimen ~30 cm in length (Fig. 3.3A) the basal-most branches are ~78% of the length of the longest branch. The following description provides a model of the anatomy of *Arborea*.

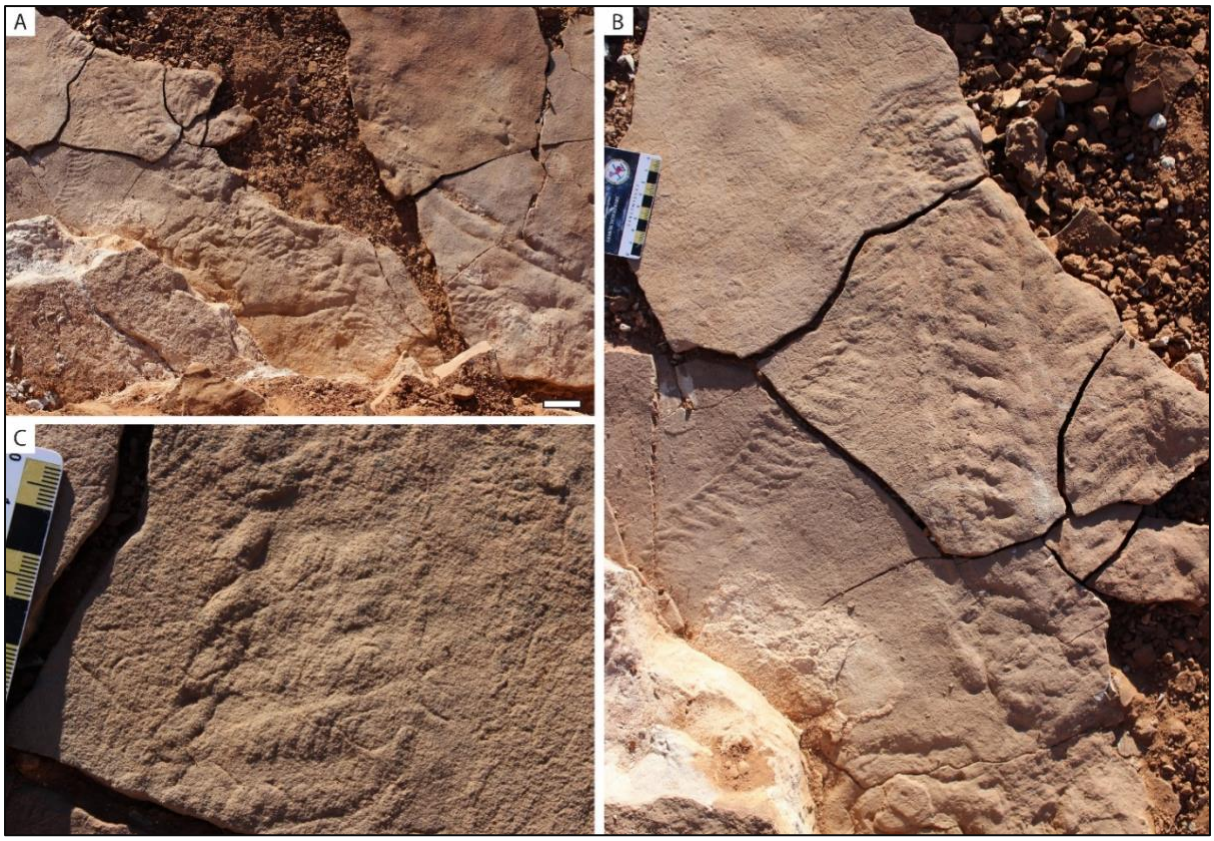


**Figure 3.2:** Large *Arborea* specimen from Nilpena, South Australia, preserved in positive epirelief. This specimen remains in the field. **A)** View of entire specimen. **B)** The distal portion of the frond appears to be folded such that only one row of lateral branches is visible (at left).

Multiple *Helminthoidichnites* trace fossils occur on the same surface. **C)** Detail of the branching towards the distal tip of the frond. Scale bar in A = 10 cm.

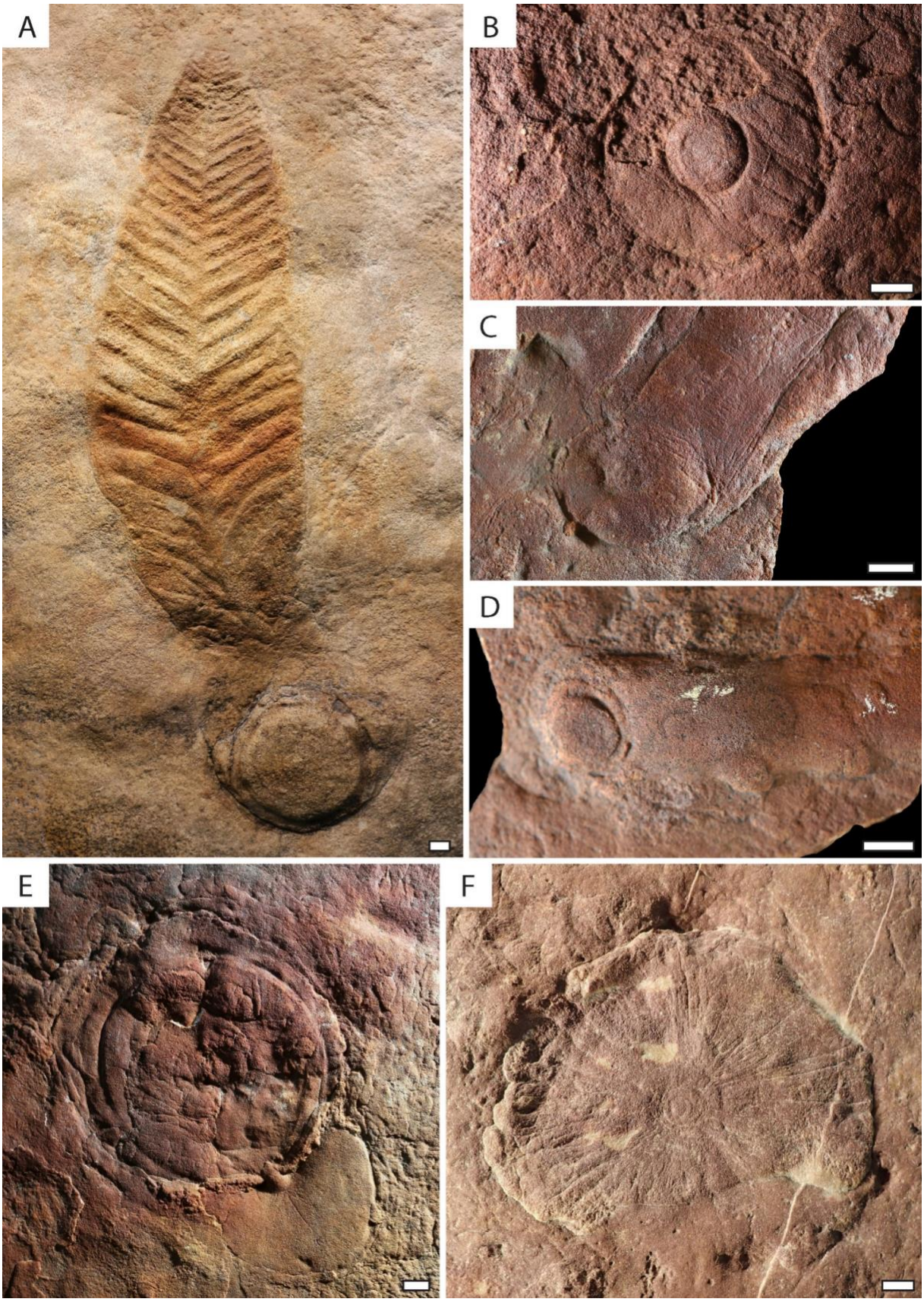
A
B
C
D
E
F

**Figure 3.3:** *Arborea arborea*, showing variability in the size and shape of *Arborea* holdfasts. All figured specimens are preserved in positive hyporelief. **A)** Complete specimen SAM P19690a, with an articulated holdfast. **B)** SAM P12888, with a single central boss and a stem whose width < the holdfast diameter (stem crosses over the holdfast at 5 oclock). **C)** SAM P40332, holdfast with a stem with width = holdfast diameter. **D)** Unlabelled specimen '52', holdfast with a stem of width ≥ holdfast diameter. **E)** Large holdfast, seemingly showing a fan of sediment (bottom right) emerging from the holdfast interior, SAM P40309. **F)** Holdfast of a large frond (SAM P49366), with radially arranged striations. Scale bars = 10 mm.


**Figure 3.4:** The 'sidedness' of *Arborea*. **A)** SAM P40785, the smallest specimen studied, with no visible sub-division of lateral branches. **B)** SAM P19690b, the tip of the frond is over-folded revealing the two sides of the organism – the bottom of the frond shows 'pods' and units, and the tip of the frond (over-folded section) shows undifferentiated rectangular branches with no visible 'pods' or units. **C–D)** SAM P34499 and SAM P35704b respectively, exhibiting smooth rectangular undifferentiated branches interpreted as the 'back' of the organism. **E)** SAM P48727 with lateral branches visible in one of the smallest described specimens. **F)** SAM

P42686, ‘pods’ and units clearly visible (interpreted as the ‘front’ of the organism), with rectangular undifferentiated branches absent. Scale bars = 10 mm.

*Arborea* possesses a holdfast structure that may variously exhibit a small number of concentric rings (Fig. 3.3A, D), a prominent but smooth central boss (Fig. 3.3B; Fig. 3.5), or multiple radial grooves (Fig. 3.3F). Such structures have, when found in isolation, previously been referred to discoidal taxa such as *Aspidella* or *Eoporpita* (Wade 1972; Tarhan *et al.* 2017), but those are now largely interpreted as organ taxa, with much of the observed variation in discoid morphology asserted to be taphonomic in origin (Tarhan *et al.* 2015; Burzynski *et al.* 2017). The holdfast connects at its centre to a single stem (Fig. 3.3), and varies in size relative to the width of the stem within the studied population, being of roughly equal diameter in some specimens (Fig. 3.3C–D), or 3–4 times larger in others (Fig. 3.3F). This variation does not appear to be directly correlated to specimen size (here measured as frond length), with a specimen of ~30 cm in length (SAM P19690a; Fig. 3.3A) possessing a holdfast of 108.6 mm diameter, while another >>74.45 cm (SAM P40858) possesses a holdfast of only 82.2 mm diameter. In one specimen, a holdfast is associated with an overlying (stratigraphically) arcuate fan of sandy material (Fig. 3.3E). This fan does not exhibit any of the known morphological characters of frond holdfasts (e.g. a central boss, or radiating striations), and a narrow projection of sand appears to connect it to the base of the disc. This relationship is inconsistent with two overlapping discs. Together with its distinct morphology, this leads us to believe that this fan does not reflect the impression of a second holdfast but, instead, represents fluid emanating from a break in the wall of the holdfast and liquidising surrounding sediment, or fluidised sediment escaping directly from the holdfast. The sediment fan is similar in morphology to lobate structures produced by fluid escape in late Ediacaran mat-bound sedimentary units in England (e.g. the Longmyndian Supergroup; Menon *et al*. 2016).

**Figure 3.5:** Multiple *Arborea* fronds, stems and holdfasts from Bunyeroo Gorge, Flinders Ranges, South Australia.

Within the studied population, the stems can exhibit variable relative lengths (see Fig. 3.3A for a very short example, or Fig. 3.3C-D for incomplete but longer examples), an observation that in other taxa has been considered functionally significant in terms of ecological tiering (Laflamme, Flude and Narbonne 2012) or reproduction (Mitchell and Kenchington 2018). Stem length shows no clear relationship to frond size. Stems can either be smooth (Fig. 3.3D), finely wrinkled (Fig. 3.3C), or have numerous grooves and interspersed ridges that run parallel to the axis of stem (Figs 3.4F, 3.6). These structures distally taper in width, and do not branch or amalgamate within the stalk, but may overlie each other (Fig. 3.6A-B). They do not continue into the holdfast in any studied specimen, and appear to record tubular structures extending up the stalk (Fig. 3.6). Along the length of the frond, the outermost tubes bend and successively exit the stalk and become the primary axes for individual lateral branches (e.g.

Fig. 3.6A-B). The tubes can appear to connect to branches either at the margin of the stalk (Figs 3.6A, C, 3.7E), or closer to its centre (Fig. 17D).

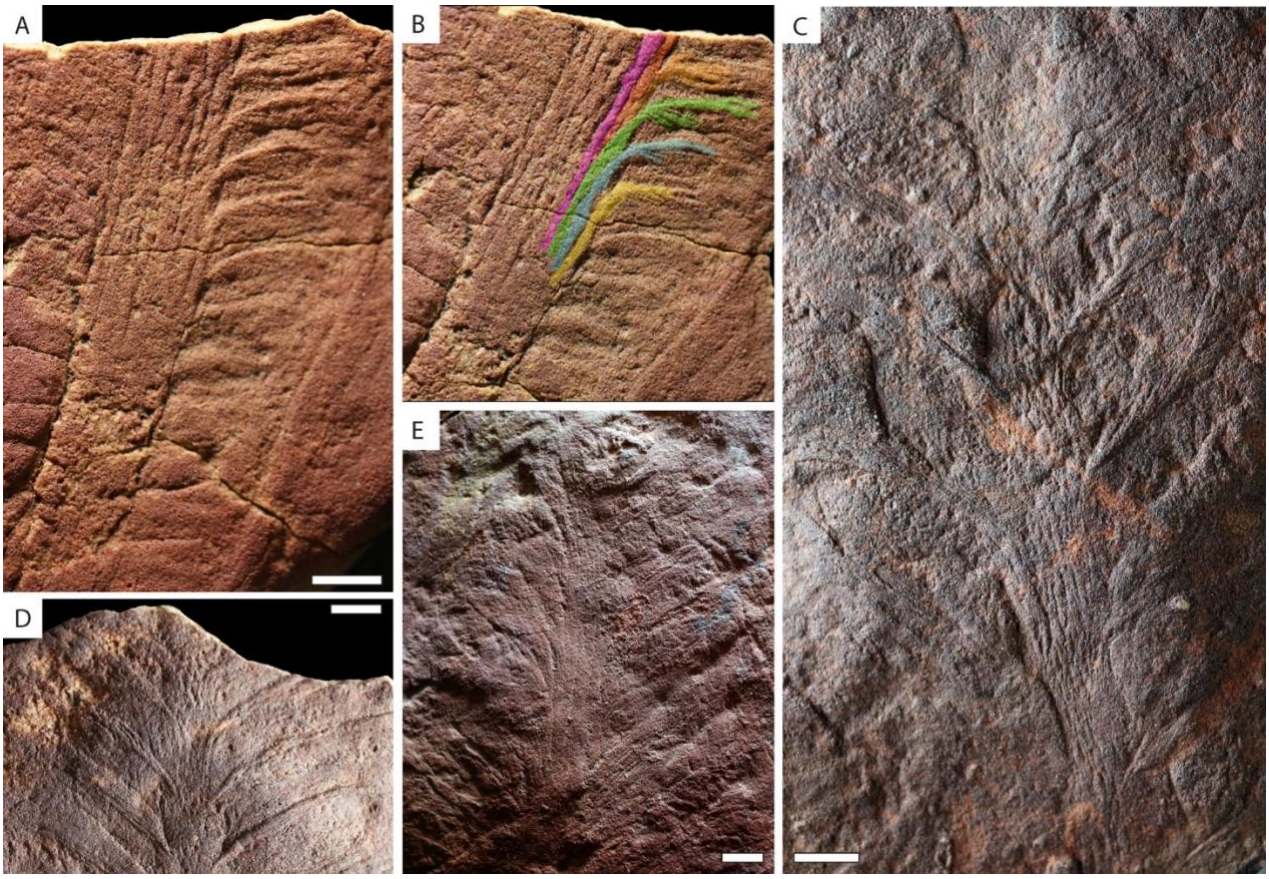


**Figure 3.6:** The fascicled arrangement of branches in the stem of *Arborea arborea*. **A–B)** SAM P47800, individual tubular structures in the stem. **A**) Tubular structures connecting in a one-for-one relationship to individual lateral branches, highlighted in B). These branches then either de-bundle or branch within the individual lateral branch. **C–E)** SAM P13801, SAM P47799 and SAM P51200 respectively, exhibiting the fascicled arrangement of tubular structures running up the stem and into individual lateral branches, where they divide further. Scale bars = 10 mm.

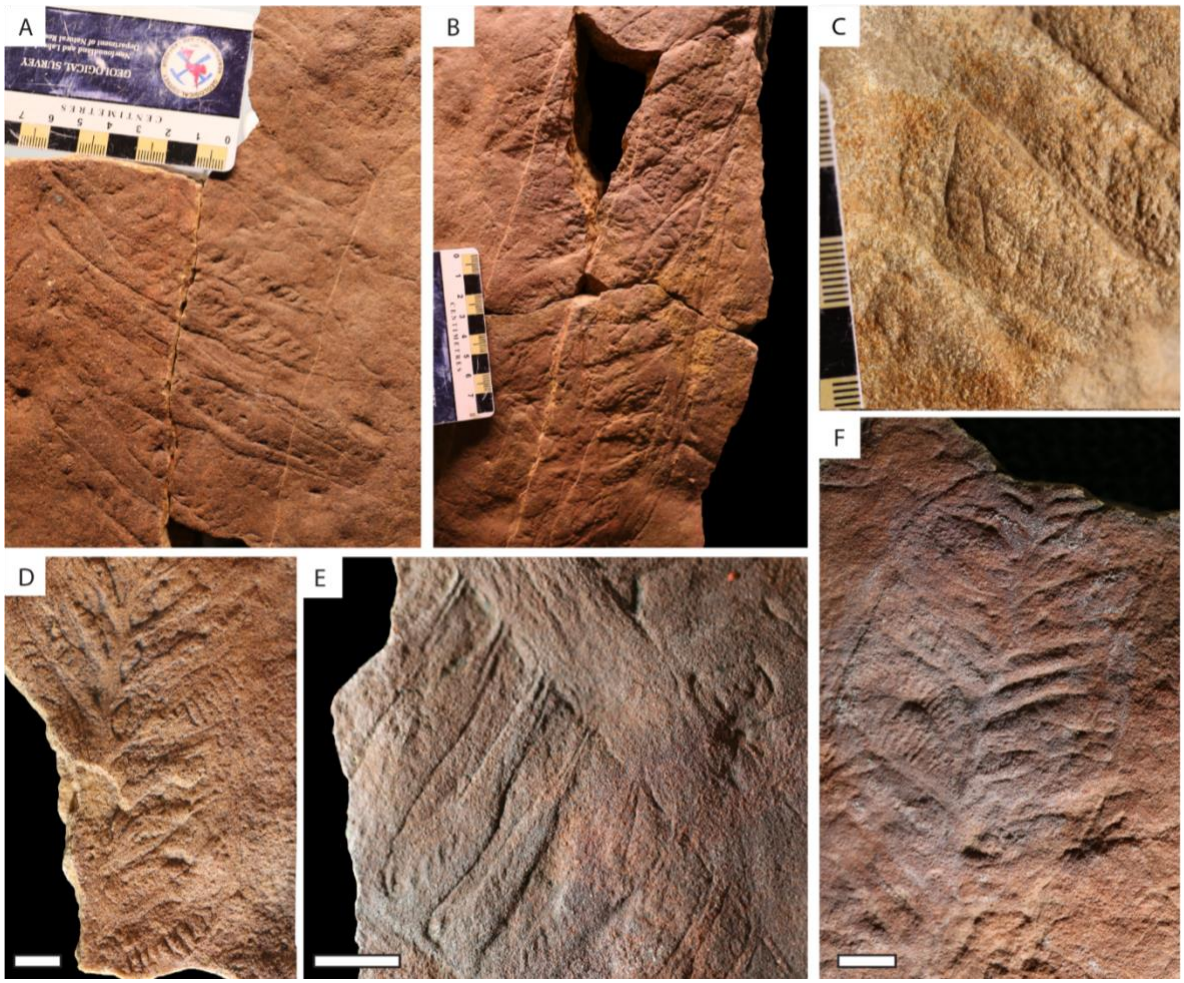


**Figure 3.7:** Detail of lateral branch morphology in *Arborea* specimens demonstrating 'pod' and unit anatomy. **A–C)** SAM P40858, with units pointing upwards in A), but downwards in B) on the opposite side of the frond (across the stalk), demonstrating that in life, units were free to pivot along the branch axis. **C)** SAM P19690b, the lateral branches with individual units showing comb-like sub-divisions. Distal at top of image in each case. **D)** SAM P40952, lateral branches exhibiting units in the absence of 'pods', specimen oriented with distal at top of image. **E)** SAM P42686, showing the connection between the 'pod' and the wide central stalk. Distal at bottom right of image **F)** SAM P40775, with units arranged on branches either side of a narrow stalk. Distal at top of image. Scale bars = 10 mm.

The frond itself is composed of two rows of lateral branches (one on either side of the central stalk; Laflamme *et al.* 2018), which appear either bilaterally or alternately arranged across the midline. The longest branches are present in the middle of the frond, with branch lengths diminishing both apically and basally (Fig. 3.3A). *Arborea* has previously been described as possessing branches resembling 'pea pods' (Laflamme *et al.* 2018), where sheet-like structures representing a continuation of the stalk wrap up and around the serially-arranged units ('peas'). Observed fronds typically show one of two possible branch variants. The first comprises solid, almost featureless rectangular blocks, which can occasionally exhibit transverse linear ornament. These abut one another to form a continuous smooth impression (e.g. Figs 3.3A, 3.4C). The second variant exhibits branches with a lenticular 'pod', partially covering a row of finely divided units (Fig. 3.7). In such cases, each lateral branch attaches to the central stalk via a single tubular structure (e.g. Figs 3.6, 3.7D–E). The distal end of the branches can also attach to the frond margin in some specimens, along what has previously been termed an undivided or marginal rim (Glaessner and Daily 1959; Jenkins and Gehling 1978). The secondary units within individual lateral branches can be oriented either apically or basally, even within individual specimens (compare Fig. 3.7A and 3.7B), suggesting that they could pivot along the lateral branch axis. In the smallest specimens, lateral branches appear bulbous, with no units visible (Fig. 3.4A). In larger specimens the units are rectangular to tear-shaped and may exhibit one order of transverse sub-divisions along their length (Figs, 3.4B, 3.7A, C; termed striations by Hoyal Cuthill and Han 2018). These subdivisions appear to emanate in a single direction, suggesting a comb-like morphology for individual units.

The tubular structures running along the stalk connect to individual lateral branches in a one-to-one, fascicled, arrangement (Fig. 3.6). They then divide and orient themselves perpendicular to the lateral branch, before branching further, or debundling, at regular intervals (Fig. 3.6A–C). Specimens only rarely exhibit both tubular structures and branch units. The tubular structures run up the lateral branches to their distal margin, dividing/debundling as they go to correspond in a one-for-one relationship with the expected positions of individual units that sit within the 'pod' (Figs 3.6A, 3,6C, 3.8A). The 'pods' never appear rectangular, as with the undifferentiated rectangular branches.

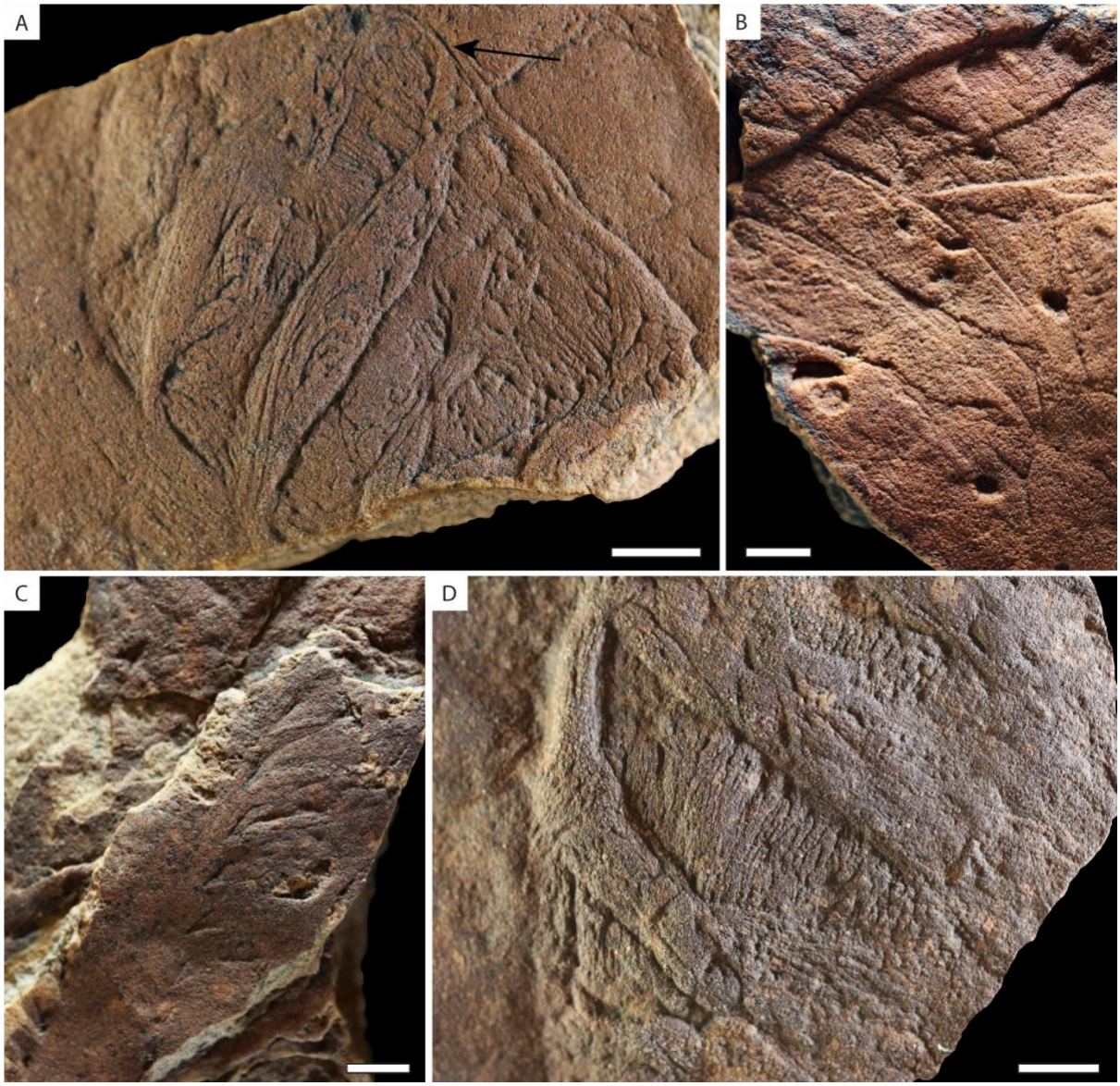


**Figure 3.8:** The backing sheet and lateral margin of *Arborea*. **A)** SAM P40786, with lateral branches splitting off the stalk (at left), but also connecting to the lateral margin (arrowed). Linear striations running apico-basally between and seemingly beneath the lateral branches may indicate the presence of a wrinkled backing sheet underlying the branches. **B)** SAM P40772, exhibiting a striated surface, interpreted as the backing sheet, in between the lateral branches. **C)** SAM P40369, individual branches connecting to a lateral margin (at right). **D)** SAM P40773, revealing a striated backing sheet between the relatively smooth lateral branches. Scale bars = 10 mm.

The lateral branches may additionally be underlain by a set of unidirectional linear striations arranged parallel (e.g. Fig. 3.8A, D) or oblique (Fig. 3.8B) to the marginal rim. These are expressed between the lateral branches, and can be present across the entire width of the frond between the stalk and the lateral margin. This striated fabric is interpreted to be a continuous sheet-like structure.

## 3.4 Discussion:

### 3.4.1 Model of anatomy

Holdfasts are rarely preserved in association with complete *Arborea* fronds, most probably due to both the large size of *Arborea* specimens, and because in life much of the holdfast would have been located beneath the sediment-water interface, and thus in a different plane of preservation (although preservation varies between beds; see Fig. 3.3A; Fig. 3.5 for examples of fronds and holdfasts preserved in the same plane). In the three clearest examples within the studied collection where the complete frond and holdfast disc are articulated, there is no relationship between the size of the frond and the size of its associated holdfast, although the smallest specimen does possess the smallest holdfast structure. Laflamme *et al.* (2018) referred to one holdfast specimen (their fig. 2.2) as 'deflated'. My observation of variable holdfast size is consistent with this interpretation. The ability of holdfasts to deflate, may be consistent with the organism being able to control and modify its shape. This interpretation is supported by the specimen with a fan of what appears to be escaping sediment (Fig. 3.3E), which may imply fluid fill within such holdfasts, and thus a potential ability to hydrostatically control holdfast size. Jenkins and Gehling (1978) suggested that there may be evidence for circular musculature in *Arborea* holdfast structures, but in the absence of further data, contraction due to dehydration remains possible (although perhaps unlikely, given the lack of contraction rims or disturbed sediment around specimens).

The stalk of *Arborea* was not blade-like, and may have been originally cylindrical (Laflamme *et al.* 2018), as supported by observed variation in the position of branch connection points, and the presence of both alternating and bilaterally symmetrical branch arrangements amongst the studied population. However, I consider at least some of this variation to result

from rotation of the branch connection points out of the plane of preservation prior to compression of the cylindrical stalk, followed by their composite molding on to the stalk in their 'rotated' positions. It is difficult to determine whether lateral branches were originally arranged in an alternating or bilaterally symmetrical manner, since these two branching arrangements are observed in almost equal numbers within the studied population.

The fascicled arrangement of tubular structures in the stalk and within the lateral branches (Fig. 3.6) appears to document the connection of individual units along each branch to the central part of the organism. These tubular structures extend into the stalk beyond the position expected of branches, and since *Arborea* is only known to possess two rows of branches, I do not consider the tubes to represent overprints of other lateral branches. The consistent one-for-one relationship of the tubes with individual lateral branches in multiple specimens precludes taphonomic interpretations such as wrinkling of an epithelium or a similar soft-tissue structure. It is not currently possible to determine whether these tubes were originally hollow or solid structures.

Since the tubular structures are most commonly observed when the pods and units assumed to reflect the exterior surface of the lateral branches are not preserved, I interpret the tubes to reflect internal anatomical features. The relatively sharp boundary between these tubular structures and the smooth stem in some specimens (e.g. Fig. 3.6) indicates that this difference is unlikely to be taphonomic in origin. These differences between the smooth exterior of the stalk and these internal structures which abut the exterior of the organism (Fig. 3.4C, 3.6A, C) may suggest that they originally comprised different materials.

The tubular structures I report were documented and termed spicules by Glaessner and Wade (1966; see also Jenkins and Gehling 1978), with that interpretation focusing on their sharp outlines and straight trajectories. However, their preservation as impressions rather than as biomineralised structures, the observation that they bend to extend into the branches, the presence of examples that curve and are clearly not straight within the stalk, and their ability to divide within the lateral branches (Fig. 3.8), lead us to question this hypothesis. True spicules in extant poriferans and cnidarians exhibit a variety of form. In cnidarians, calcitic spicules represent a derived condition, being present only in the Octocorallia. They are

secreted by the mesoglea, and are largely concentrated in the base of the colony, but may also be present in polyp leaves, or on anthocodia (Hyman 1940). In siliceous sponges, spicules are generally classified as either microscleres (smaller 'flesh' spicules), or megascleres (the main skeletal support elements). Megascleres are known to reach sizes of up to three metres (and be up to 8.5 mm in diameter) in the basalia of *Monorhapis chuni*, where they function as a stalk (Müller *et al.* 2007). More commonly, microscleres are on the order of 1–60 µm, whereas megascleres are between 60–200 µm, and both can bundle and inter-weave (e.g. in the order Halinchondrida; Hooper 2004). The continuation of tubular structures up the stalk of *Arborea* and into its individual branches and units is an arrangement not seen in any extant spicular organism.

An alternative possibility, favoured here, is that the tubular structures in *A. arborea* represent non-mineralizing, stolon-like projections, consistent with their length, seemingly flexible nature, and one-to-one relationship with individual lateral branches, and then units (Fig. 3.8). Stolons or stolon-like projections represent a derived condition in the Bilateria, but are nevertheless possessed by several invertebrate groups (e.g. the Bryozoa [Osborne 1984] and Entoprocta [Nielson 2012]), as well as many plants (de Kroons and Hutchings 1995) and algae (Ceccherelli, Piazzi and Balasta 2002), while fungal mycelia (Benjamin and Hesseltine 1949) may also produce thread-like projections. Horizontal creeping stolons are known in many land plants (e.g. *Fragaria ananassa*; Savini *et al.* 2008) and in algae (e.g. *Caulerpa prolifera*; Ceccherelli *et al.* 2002). In the siliceous and calcareous sponges, stolons can take a variety of forms, including creeping stolons (e.g. the calcarerous sponge *Leucosolenia* [Padua and Klautau 2016]) and reinforced structural stolons (e.g. the carnivorous demosponge *Chondrocladia lyra*; Lee *et al.* 2012). Poriferan stolons are not known to bundle. Cnidarian clades exhibit stolons whose morphological variation encompasses horizontal creepers, and (particularly in the Hydrozoa) bundled vertical projections (Schuchert 2001), or fascicles. These fascicles may surround a 'true' stem but be encompassed by periderm (e.g. in the hydrozoan *Plumularia*), or may themselves comprise the stem (e.g. in the hydrozoan *Eudendrium;* Hyman 1940 fig. 116). Such fascicled branches provide the most similar extant analogue for the arrangement of tubular structures seen in *A. arborea*.

If the holdfast of *Arborea* was hydrostatically regulated, some form of hydraulic system would be expected. I find no evidence for any such system, but note that some extant hydraulic systems, such as the inhalant and exhalent siphonozooids of pennatulaceans (Williams, Hoeksema and van Ofwegen 2012) are unlikely to preserve in Ediacaran frondose taxa due to their position beneath branch attachment points along the stalk. Alternatively, the fascicled tubes may have been involved in hydraulic regulation, particularly if the individual units to which they connect were open to the water column.

The 'backing sheath' in *Arborea* – the apparent connective structure that joins the stalk with the marginal rim – may have anchored the lateral branches in place, though Laflamme *et al.* (2018) propose that the rim could alternatively reflect folding of the distal tips of the lateral branches. The Russian frondose taxon *Charniodiscus yorgensis* has also been interpreted as having first-order branches that are constrained along their horizontal axes, but unlike *A. arborea*, *C. yorgensis* is reconstructed as exhibiting full branching units on both sides of the organism (Ivantsov 2016). No fascicled branching arrangement has been noted in *C. yorgensis* despite the pyritization of internal anatomical features.

The observation that 'pods' and units can be present or absent in *Arborea* specimens, even within individual specimens (Fig. 3.4B), is consistent with the suggestion that they are only present on one side of the organism, conferring front-back distinction (Fig 3.9; Jenkins and Gehling 1978; Laflamme *et al.* 2018). The 'back' of the organism comprises the backing sheath, subdivided into rectangular blocks defined by lateral seams. The linear striations observed running behind lateral branches in certain specimens (e.g. Fig. 3.8A) are interpreted to reflect either the inner surface of the backing sheath, or a distinct layer within the organism. In addition to the clear apico-basal differentiation of the organism, this character can potentially assist in constraining phylogenetic affinities in conferring two main body axes on the organism.

Lateral branches were attached to the stalk by both a tubular continuation of external tissue, and by the internal tubular projections (leading to apparent pairing of connections in some specimens; Gehling 1991). Lateral branches consist of two main elements: the 'pod', which was constructed of two lens-shaped sheets (not bound to each other at either their apical or

basal margins; Fig. 3.9), and the sub-rounded to comb-shaped units (Fig. 3.9 inset), which lay within the pod. Previous studies have considered subdivisions within second order units to reflect wrinkling of a soft tissue structure (Laflamme *et al.* 2018), but their consistent morphology both within and across specimens leads us to consider them biological features. I note that the lateral branches of *Arborea*, being comprised of a lenticular 'pod' and subdivided units housed therein, differ fundamentally in architecture to the linear subdivisions seen in second and third order units. This distinction does not fit the 'self-similar' branching definition of the Rangeomorpha, and I therefore follow previous workers (e.g. Laflamme and Narbonne 2008a) in considering branching arrangements in *Arborea* to be distinct.

If the pod does indeed surround the units, this has potentially interesting implications for the production of micro-eddies and flow disturbance around the units (which have previously been hypothesised to explain community dynamics in Ediacaran fronds; Singer, Plotnick and Laflamme 2012; Ghisalberti *et al.* 2014), potentially aiding nutrient uptake in these regions. Laflamme *et al.* (2018) noted consistencies between *Arborea* morphology and morphology required for feeding in extant pennatulaceans.

The anatomical arrangement I describe is distinct from both the fractal rangeomorphs (Narbonne 2004) which diagnostically require three orders of identical branching (Erwin *et al.* 2011), and also from the latest Ediacaran erniettomorph *Swartpuntia germsi*, which is characterised by a multi-vaned arrangement of featureless tubular branches (Narbonne *et al.*, 1997). Recent studies suggesting a close phylogenetic relationship between the morphogroups Rangeomorpha, Arboreomorpha and Erniettomorpha (Deccechi *et al.* 2017; Hoyal Cuthill and Han 2018) do not find support from our re-analysis of the anatomy of *Arborea*.

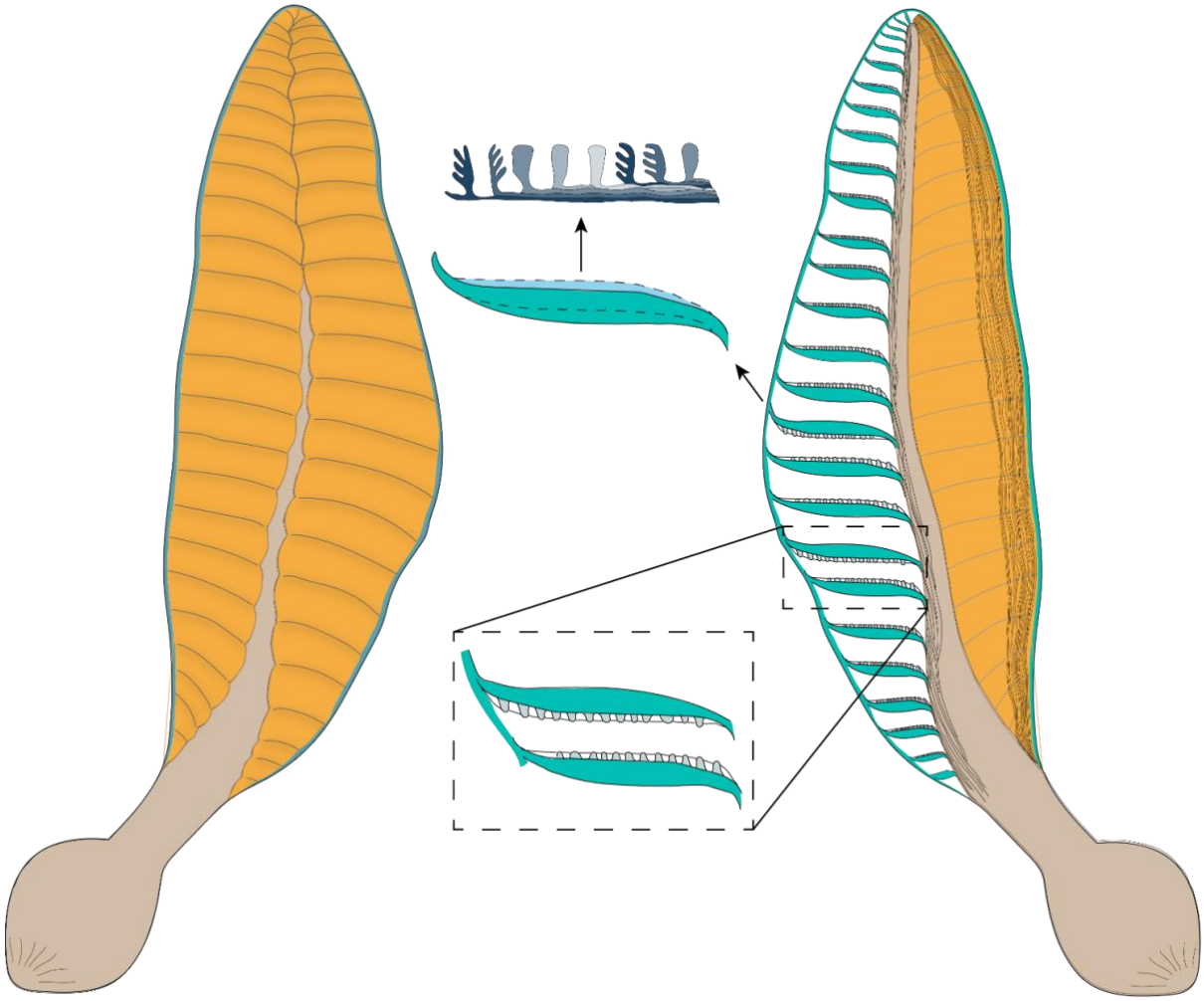

**Figure 3.9:** An anatomical reconstruction of the Ediacaran frondose taxon *Arborea arborea*, based on the features discussed in this chapter. The 'back' (left) and 'front' (right) faces of the organism are shown. The right-hand side of the front shows the organism with the 'pods' and units (i.e. the branches) removed to reveal the underlying backing sheet. Inset: fine-scale arrangement of units within the 'pod'. Units are each connected to their own tubular, stolon-like structure running into the stalk. Note that pods (green) are free to pivot about the lateral branch axis, and that pod margins are not connected apically or basally (separate pod 'sheets' are indicated by a full line and a dashed line respectively in inset).

### 3.4.2 Growth

The anatomical organisation described above permits inference on the morphogenetic strategy of *Arborea*, which is informative when considering organismal affinities. The smallest, assumed to be least developed, specimens of *A. arborea* possess fewer branches than larger specimens. This suggests that branch growth and differentiation actively occurred during the frondose stage of the organism's life cycle, with new tubular structures presumably developing and terminally differentiating (based on the consistently smaller size of apical branches) as the frondose organism grew (rather than undergoing a single event of terminal differentiation). I find no upper size limit to *Arborea*, and thus suggest that it may reasonably be interpreted to have displayed indeterminate (size) growth, with no known maximum number of branches. Significant branch differentiation appears to have occurred in small specimens, with the smallest known specimens (~3.5 cm) already possessing ~19 lateral branches. *Arborea* also shows a determinate (i.e. consistent and predictable) form within the studied population, with no evidence for aberrant branches (branches that are unusually long or short, or do not conform to the expected branching architecture; e.g. Kenchington *et al.,* 2018). That the frond outline appears to change as specimen size increases, with the basal-most branches becoming relatively larger despite continued branch differentiation, suggests that new branches in *Arborea* differentiated from a (sub)apical generative zone (as indirectly inferred by Hoyal Cuthill and Han 2018). I find no evidence for further, lateral generative zones.

An ordered fascicled branching arrangement requires a unidirectional guidance and pathfinding system along both the apico-basal and front-back axes. Pathfinding refers to the ability of a cell or group of cells to locate their final destination: neurons, for example, are able to find their destination by growing in permissive substrates, and binding to adhesive cues (Raper and Mason 2010). Differentiation of the tubular structures into both branches and units occurs only after they emerge from the stalk wall, suggesting either the removal of an inhibitory signal within the stalk, or the presence of a positive differentiation signal in the stalk wall. The strategy outlined above is consistent with morphogenesis of branches in *Arborea* having occurred by localised outgrowth, as opposed to regional apoptosis (from an undifferentiated sheet). This is in line with many other forms of branching growth in extant

eukaryotes, for example that seen in the alga *Ectocarpus* (Katsaros, Karyophyllis and Galatis 2006), or the bilaterian tracheal network (Affolter, Zeller and Caussinus 2009).

### 3.4.3 Phylogenetic inference:

It is reasonable to assume that the anatomical complexity and large size of some *Arborea* specimens (~3m in length) demonstrate that it was a multicellular organism, dwarfing even the largest multinucleate protists (xenophyophores). Indeterminate growth is compatible with several non-metazoan (e.g. Peterson, Waggoner and Hagadorn 2003) and metazoan (e.g. Sebens 1987) hypotheses of affinity, and is thus not considered an informative character here. *Arborea* lacks (e.g. Jenkins and Gehling 1978; Laflamme *et al.* 2018) the serially quilted arrangement that has been considered diagnostic of the Vendobionta, and inferred in some rangeomorph taxa (Seilacher *et al.,* 2003; Seilacher 2007). The constrained form of *Arborea* within populations exhibits no aberrant branches (branches that are unusually long, or unusually short), a lateral margin bounding the branches, and determinate changes in form (i.e. a transition from a fusiform to a distally tapering frond outline). This is inconsistent with the growth pattern of many extant modular groups (e.g. plant or algal groups). The differentiation of new branches as *Arborea* grew is also incompatible with a fungal affinity, where a fruiting body undergoes one round of terminal differentiation (Umar and Van Griensven 1997). I therefore consider that to the exclusion of extant non-metazoan comparators, *Arborea arborea* was a total group metazoan.

The constrained form, presence of two main body axes, and extensive body regionalisation is incompatible with a poriferan affinity, but such an axial arrangement is compatible with a eumetazoan affinity. I recognise differential preservation of anatomical features in *Arborea*, with structures in the interior of the organism moulded on to the exterior, or external structures being entirely or partially missing in different specimens. This may suggest that these structures were distinct, and potentially composed of different original materials. If correct, this could indicate tissue differentiation: a eumetazoan character. Possession of a fluid-filled holdfast, potentially indicating a capacity for hydrostatic regulation, is also compatible with, but not unique to, a eumetazoan affinity. On the basis of all available evidence, I therefore propose that *A. arborea* lies within the total- group Eumetazoa. Such a

phylogenetic position has been presented previously (e.g. Buss and Seilacher 1994; Hoyal Cuthill and Han 2018; though I disagree with the clade of Ediacaran organisms favoured by these authors), but this reassessment of *Arborea* provides developmental and anatomical support. Our current knowledge of anatomical characters in *Arborea* is insufficient to permit further constraint of its phylogenetic position.

The fascicled internal anatomy of *Arborea* suggests that each lateral branch grew independently of its neighbours, implying developmental independence and thus conforming to the definition of biological modularity. Such an arrangement is comparable with extant taxa that possess colonial organisation (e.g. various hydrozoans; Hyman, 1940), and it is therefore entirely feasible that *Arborea* could represent an Ediacaran colonial eumetazoan (*contra* Landing *et al.* 2018). Coloniality has previously been predicted to be the plesiomorphic condition for the Cnidaria, with *A. arborea* itself (then termed *Charniodiscus*) proposed to lie at the base of the cnidarian tree (Dewel 2000; see also putative stem-group colonial cnidarians from Cambrian Series 3; Park *et al*. 2011). However, more recent work (Zapata *et al.* 2015; Kayal *et al.* 2018) would suggest that this scenario is unlikely, with coloniality only being known in derived cnidarian positions. Ctenophores are not known to be colonial (I favour the view that Porifera represent the earliest diverging animal clade; Simion *et al.* 2017; Fueda *et al.* 2017), suggesting that the Ur-eumetazoan was a unitary organism. Coloniality is also noted as a derived condition within the Bilateria, with the only truly colonial phylum being the Bryozoa. If our interpretation of *Arborea* as a potentially colonial organism is correct, this may suggest that coloniality in eumetazoans was present in early-diverging groups. With no current evidence to tie *Arborea* to any crown groups, this character could feasibly be present in early-branching positions of the eumetazoan stem-lineage, suggesting further (perhaps derived) excursions into the colonial state were possible, broadening the possible permutations of the eumetazoan ancestor.

## 3.5 *Arborea arborea* from Charnwood Forest?

*Charniodiscus concentricus* – the first named arboreomorph – was first recorded in Charnwood Forest in 1958 as an organ taxon as a circular disc, erroneously considered part of the frondose form *Charnia masoni* described in the same paper (Ford, 1958). The *Charniodiscus* frond was later described, but once again considered to belong to the taxon *Charnia masoni* (Ford, 1962). The frond of the organism was not described as a different organism until 1978 (Jenkins and Gehling 1978). However, since that time very little work looking at the arboreomorphs of Charnwood Forest has been conducted, excepting the holotype itself (Dzik 2002; Antcliffe and Brasier 2009), despite the recognition of ~70 specimens of arboreomorph fronds (Wilby, Carney and Howe 2011).

Unlike the shallow-water Ediacara Member of South Australia, arboreomorph bearing rocks from Charnwood Forest record a deep-water turbiditic environment, and are dated at between 557 and 562 Ma (Noble *et al.* 2015). As such, they are considered to host fossils of the Avalon biotic assemblage, as opposed to the White Sea assemblage of South Australia, and predominantly record rangeomorph taxa, which numerically constitute at least 27% of the total fossil assemblage, and make up 60% of total fossil diversity (Wilby *et al.,* 2011). This is unlike the communities recording *A. arborea* in South Australia, where specimens may be found alongside evidence for motile metazoans such as *Dickinsonia*, or the furrow and levee trace fossil *Helminthoidichnites* (Gehling and Droser 2013).

A number of specimens of frondose organisms from Charnwood Forest, previously referred to ‘arboreomorph undet.’ (Wilby *et al.,* 2011; Kenchington *et al.* 2018), show clear anatomical similarities to *Arborea* (see Fig. 3.9), and the affinity of these specimens is reviewed herein. I conclude that these specimens should be referred to *Arborea* (Laflamme *et al.* 2018), thus extending both the stratigraphic range and the known environmental tolerances of the genus *Arborea.*

### 3.5.1 Materials and methods

While there are ~70 specimens of arboreomorph specimens, most conform to the definition of genus *Charniodiscus* (Antfliffe and Brasier 2009; Laflamme *et al.* 2004; Laflamme *et al.* 2018). However, three specimens ascribed to the arboreomorphs do not conform to this definition, and are examined here. The three specimens are all preserved in lateral aspect from Bed B of the Bradgate Formation, Maplewell Group, Charnwood Forest, UK. Specimens were not retrodeformed as they are current aligned, and so are assumed to have undergone a similar level of deformation (Wilby *et al.* 2011).

### 3.5.2 Results

In this section, I describe two rare morphs of 'arboreomorph undet'.

Specimens may possess a large disc, which is connected to a bifoliate frond via a stem (Fig. 3.10). A complete specimen is ~29cm in length, and is associated with a disc of ~13.9 cm diameter (Fig. 20). The frond itself possesses multiple first order branches, which are bound medially to a central stalk, and laterally by a smooth and contiguous distal margin which defines the outline of the frond. The first order branches are arranged in a bilateral to glide symmetrical arrangement, and are rectangular to sinusoidal (Figs 3.10 – 3.11). The branches at/near the apex of the frond are the shortest in absolute length (subapical branches measure ~20mm, but the most apical branches are too poorly preserved to quantify). The most basal branches preserved are 41 – 44mm in length, and branches are consistent in length up to the mid-frond (all measured data derived from specimen GSM 105960).

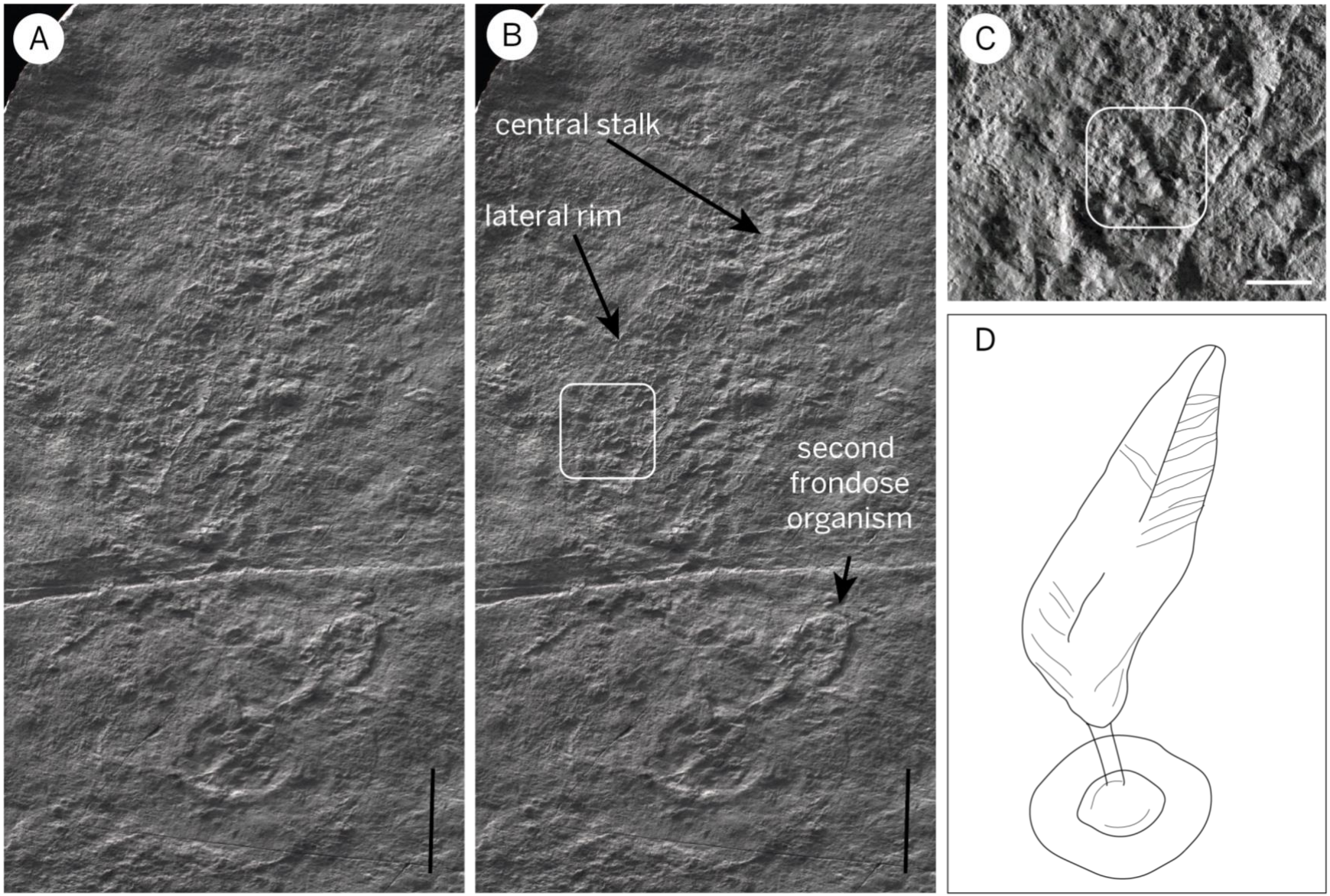


**Figure 3.10: A)** *Arborea* specimen from Charnwood. Forest, UK, specimen GSM105960. **B)** labelled version of specimen in **A**. **C)** second and third order branches interpreted as 'units'. The white box refers to the boxed area in **B**. **D)** schematic representation of features in A and B. Scale bar in A, B = 5cm, in C = 1cm.

Units are observed as connected to the basal margin of a sinusoidal lateral branch, and may appear subdivided (Fig. 3.10C). In these instances, both first and second order branches are oriented in a single direction.

Rectangular lateral branches (Fig. 3.11) on one face of the organism are of consistent width and exhibit a glide symmetrical arrangement. The medial margins of these branches may appear as a single 'seam' (Fig. 3.11A-C). This is unlike the medially tapering margins of the sinusoidal branches on the presumed front, which connect to a central stalk. A stalk is sometimes visible beneath the rectangular branches (Fig. 3.11A-B), but branch margins do not meet the margins of the stalk-like structure. Specimens exhibiting rectangular branches may also possess a raised distal margin (Fig. 3.11D-E). Rectangular (first order) branches may bear faint impressions of consistently spaced second order branches (Fig. 3.11C).

One specimen appears to exhibit a second, smaller fond overlying its holdfast (Fig. 3.10A-B). First order branches, stretching from a central axis to the margins of this frond are visible, but no further comments on branching architecture can be made. The frond is sub-rounded, with a linear basal margin and naked stem (Fig. 3.11A-B). This is unlike the adjacent larger frond. The relationship between these fronds, if any, is not clear.

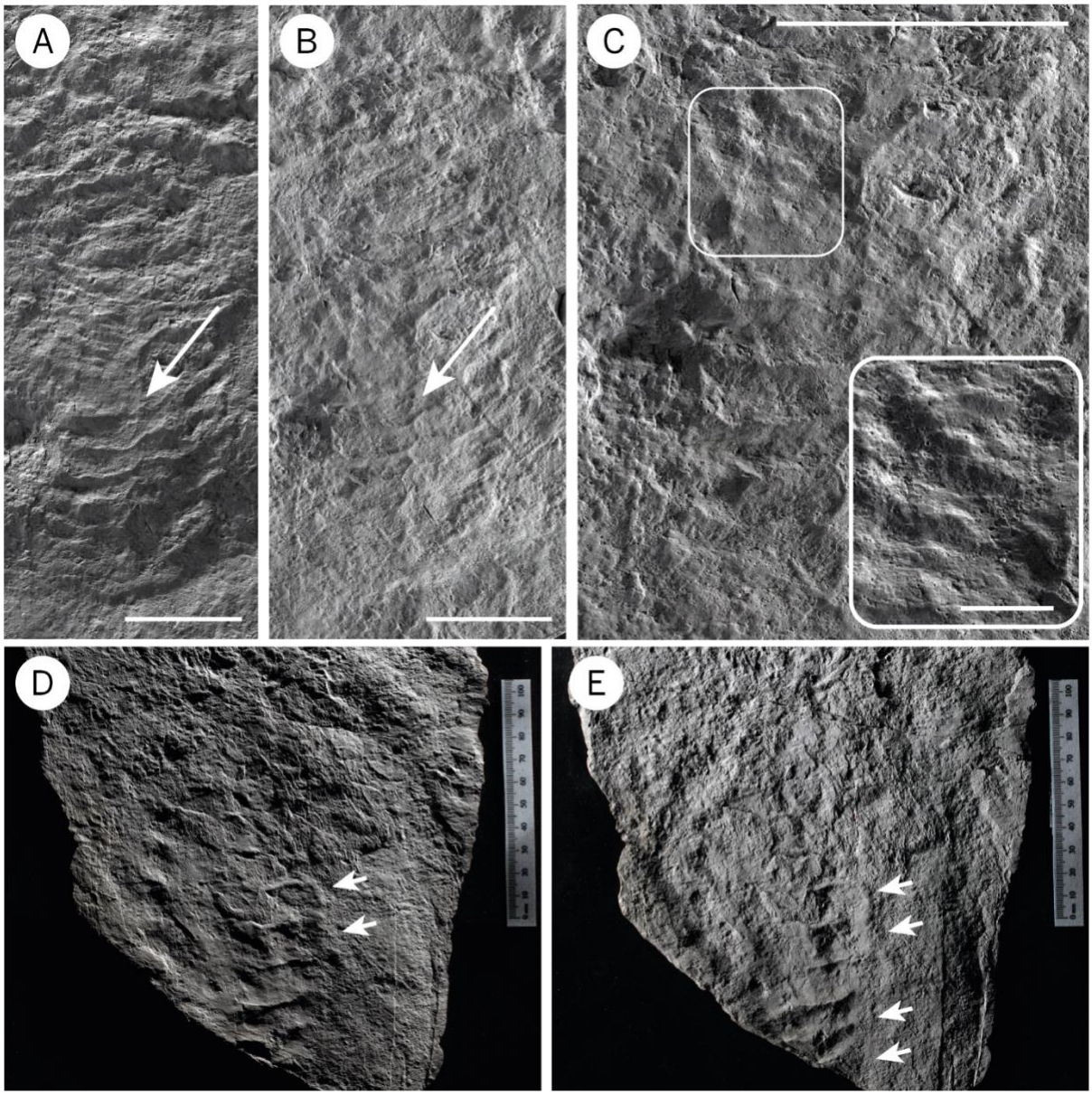


**Figure 3.11:** The 'back' of *Arborea* from Charnwood. **A – C**) Specimen GSM106024 (viewed under different lighting) with **A-B** showing the glide symmetrical meeting of rectangular first order branches, with faint stalk visible underneath. Linear stalk-like margin with overlying first order branch margins arrowed in **A** and **B**. **C** the units within rectangular first order branches; boxed area, highlighting second order branches, enlarged. **D–E** GSM cast 629 (viewed under lighting from different angles) with rectangular first order branches flanked by a lateral rim (white arrows). A, B, C, scale bar = 5cm, inset in C, scale bar = 1cm.

### 3.5.3 Discussion

I interpret the associated disc structure in specimen GSM105960 as a holdfast, as is the case with many described disc structures associated with Ediacaran frondose organisms (e.g. Burzynski and Narbonne 2015; Burzynski *et al.* 2017). Apically-oriented branches may be present in the specimens with sinusoidal branches, and in the specimens with rectangular branches. Both branching forms may exhibit a contiguous raised lateral margin. This combination of separated apically-oriented branches and a contiguous lateral margin are not found in any other taxa from Charnwood Forest. The specimens exhibiting rectangular branches may show the impression of a stalk-like structure underlying the branches (with the branch margins not associated with the margins of the stalk-like structure). This may suggest that the rectangular branches represent only one face of the organism. I consider it likely that these specimens represent different faces of the same organism.

Given the lack of morphological information available for the small frond in Fig. 3.10B, I exclude it from the following discussion.

*(a) Comparison to other frondose taxa:*

This anatomy does not conform to the definition of any known rangeomorph, which are all described as having faces which bear an identical morphology. The only rangeomorph described as possessing a raised lateral margin is *Rangea schneiderhoehni*, known to bear six faces (hexafoliate), which are all identical. I find no evidence to suggest these Charnwood specimens exhibited any more than two faces. The bifoliate nature of these fossils, along with the subdivisions present on some specimens, precludes an alliance with the terminal Ediacaran frondose erniettomorph *Swartpuntia germsii*, characterised by multiple vanes constructed of tubular units. The presence of further branched subdivisions after the first order branch also rules out comparisons with the erniettomorph *Pteridinium* (e.g. Meyer *et al.* 2014).

The only other currently described frondose components of the Ediacaran Macrobiota are the arboreomorphs, for which there are five described species; *Charniodiscus spinosus, Charniodiscus procerus, Charniodiscus concentricus, Charniodiscus yorgensis* and *Arborea arborea.*

None of the specimens described here possess an apical spine, which is required for an affiliation with *C. spinosus* (Laflamme *et al.* 2004), and the stalk (where present) does not represent the greatest proportion of the length of the organisms, required for an association with *C. procerus* (Laflamme *et al.* 2004). *Charniodiscus concentricus* has been described as possessing multiple vanes (Antcliffe and Braiser 2009), although it has been noted that a multifoliate anatomy has not been found in any other arboreomorph from Charnwood Forest (Wilby *et al.,* 2011), and this has been used as a diagnostic character (Antcliffe and Brasier 2009; Laflamme *et al.* 2018). The fossils described here display no evidence for more than two vanes, and so they may not be referred to *C. concentricus. Charniodiscus yorgensis* is described as possessing first order branches (lateral lobes; Ivantsov 2016) that reach to the lateral margins of the specimen. This is unlike the specimens described herein, where a smooth and raised lateral rim is present. Indeed, *C. yorgensis* is described as having a uniform anatomy across both faces of the frond. This does not conform to the anatomy of the fossils described here. Therefore, these fossils are not referred to *C. yorgensis.*

*(b) Arborea?*

The specimens described here do align with the current definition of *Arborea arborea*, which requires a bifolaite frond with a prominent stalk, and is described as having two distinct faces – one bearing subdivided units, and the other displaying rectangular branches that possess no further branched subdivisions (illustrated in Fig. 3.9).

The branching arrangement described here, whereby second and third order branch subdivisions are only present on one side, is also reminiscent of the branched anatomy of *Arborea*, described in section 3.3 (Fig. 3.6). I therefore assign these specimens from Charnwood Forest to *Arborea.*

This represents the first documented appearance of *Arborea* outside the Ediacara Member of South Australia. The fossils described from the Ediacara Member of South Australia are dated as ~550 million years old, and thus the description of *Arborea* from Charnwood Forest extends the known stratigraphic range by 7–12 million years. The presence of *Arborea* in Charnwood Forest also expands the environmental tolerances of this organism. The Ediacara Member records shallow-marine to deltaic depositional environments (Gehling and Droser 2013; Reid *et al.* 2018), while the Bradgate Formation records deep-water fore- or back-arc basin environments (e.g. Kenchington *et al.* 2018). Wilby *et al.* (2011) document the orientation of frondose specimens on Bed B, and show that the majority of these specimens, including the complete *Arborea* specimen described herein, are aligned. These specimens are therefore interpreted as being current aligned, tethered to the sediment and *in situ* at the time of burial. This suggests that *Arborea* was able to live at depth, out of the photic zone. Indeed, the global distribution of *Arborea* may imply, as has been hypothesised for the frondose rangeomorphs (Darroch, Laflamme and Clapham 2013), a water-borne propagule was part of *Arborea's* life cycle. However, this suggestion remains speculative at present.

*Arborea* is a rare component of the Charnwood Ediacaran deposits, and has never been described in the Avalonian communities of Newfoundland, Canada. The rangeomorph communities of Charnwood Forest are also distinct, with a number of endemic species, including the recently described *Hylaecullulus fordii* (Kenchington *et al.,* 2018). Reciprocally, some of the most common Newfoundland taxa (e.g. genus *Fractofusus;* Gehling and Narbonne 2007) are seemingly absent from the described Charnwood communities. These differences serve to highlight the diversity of Avalonian communities, the first diverse and complex macroscopic ecosystems on Earth. Indeed, it also may suggest a cryptic diversity in Avalonian deposits, linking them (compositionally) to the younger White Sea assemblages. Indeed, the interpretation of *Arborea* as a total-group eumetazoan increases the diversity of Avalonian Eumetazoa beyond the rare non-frondose taxon *Haootia quadriformis* (Liu *et al.* 2014) and supports a more diverse (and disparate) Avalonian biota.

## 3.6 Conclusions:

Reconstruction of the anatomy and developmental biology of *Arborea arborea* leads me to conclude that it likely represents a total-group eumetazoan. In addition to previously recognised morphological characters (Laflamme *et al.* 2018), I note a distinctive fascicled internal branching arrangement and a fluid-filled holdfast. The different taphonomic expressions of structures within the studied *Arborea* collection may imply the presence of different tissue types, and thus tissue differentiation. I conclude that *Arborea* was a modular organism, and note that it displays characters consistent with (but not exclusive to) a colonial body-plan, something previously argued to have emerged in eumetazoans only in the Ordovician (Landing *et al.* 2018). Key differences between *Arborea* and rangeomorphs support morphological distinction between these frondose organisms, hinting at multiple independent excursions into frondose morphospace amongst early diverging animal groups.

Furthermore, I conclude that specimens attributable to the genus *Arborea* are present in deep-water deposits. This represents the first report of an iconic 'White Sea' assemblage taxon outside shallow water assemblages. The discovery of an iconic member of the Ediacaran White Sea biota in Avalon-type deposits undermines the description of the 'three assemblages' concept as representing a series of evolutionary transitions. While *Arborea* is undoubtedly a rare component of the Charnwood Forest palaeocommunity, its presence indicates a more protracted appearance of taxa associated with the 'second wave' of the Ediacaran Macrobiota (Tarhan *et al.* 2018), and lends further support to the idea that preservational constraints play a large role in understanding the three Ediacaran assemblages.

# Chapter 4:

## The anatomy of the Ediacaran rangeomorph *Charnia masoni*:

**Author contributions:** A version of this chapter is published in *Papers in Palaeontology.*

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This study was designed by F.S.D and A.G.L. F.S.D, A.G.L, Dmitriy V. Grazdhankin and Jack J. Matthews collected the data, and F.S.D conducted all analyses. F.S.D produced the first draft of the manuscript and all authors provided feedback on the manuscript. F.S.D is the lead author of the paper. F.S.D contributed ~85% of the work presented in this chapter.

# Abstract:

The Ediacaran macrofossil *Charnia masoni* is perhaps the most iconic member of the Rangeomorpha: a group of seemingly sessile, frondose organisms that dominates late Ediacaran benthic, deep-marine fossil assemblages. Despite *C. masoni* exhibiting broad palaeogeographic and stratigraphic ranges, there have been few morphological studies that consider the variation observed amongst populations of specimens derived from multiple global localities. I present an analysis of *C. masoni* that evaluates specimens from the UK, Canada and Russia, representing the largest morphological study of this taxon to date. I describe morphological variation within *C. masoni* and present a new morphological model for this species that has significant implications both for interpretation of rangeomorph architecture, and potentially for existing taxonomic schemes. Previous reconstructions of *Charnia* include assumptions regarding the presence of structures seen in other rangeomorphs (e.g. an internal stalk) and of homogeneity in higher order branch morphology; observations that are not borne out by my investigations. I describe variation in the morphology of third and fourth order branches, as well as variation in gross structure near the base of the frond. The diagnosis of *Charnia masoni* is emended to take account of these new features. These findings highlight the need for large-scale analyses of rangeomorph morphology in order to better understand the biology of this long-enigmatic group.

## 4.1 Introduction:

The earliest known palaeocommunities of the Ediacaran Macrobiota date to ~571–560 Ma (Noble *et al*., 2015; Pu *et al*., 2016) and are found amongst sedimentary rocks deposited in deep marine palaeoenvironments (e.g. Liu *et al.* 2012; Wilby *et al.* 2015). They are dominated by organisms with a frondose body plan that could reach up to two metres in length (Narbonne and Gehling 2003; Liu *et al.* 2015). Some of these fronds exhibit self-similar (sometimes considered 'fractal') branching and have been assigned to the morphogroup Rangeomorpha (Pflug 1972; Jenkins 1985; Narbonne 2004; Erwin *et al*., 2011), which may comprise a clade (Deccechi *et al.* 2017). The constructional architecture of rangeomorphs has proven difficult to reconcile with the body plans of extant taxa, resulting in multiple competing hypotheses, including both metazoan and non-metazoan affinities, for members of the group. These interpretations have included algae (Ford 1958), fungi (Peterson *et al*. 2003), lichens (Retallack 1994), total-group metazoan (Budd and Jensen 2017) and pennatulacean cnidarians (Glaessner 1989). Assessment of developmental data derived from rangeomorphs concluded that most of these interpretations are not compatible with morphogenetic evidence, and that rangeomorphs are likely to fall within the total group Metazoa (discussed in Chapter two).

Recent field and museum visits in Newfoundland (Canada), Charnwood Forest (UK) and the White Sea (Russia) have unearthed new material that includes rangeomorph specimens of markedly different sizes within individual species. Such specimens are interpreted as different developmental stages of the organisms (Liu *et al.* 2012; Wilby *et al.* 2015) and provide new opportunities to obtain insight into both rangeomorph anatomy and morphogenesis. The prominent rangeomorph taxon *Charnia masoni* (Ford 1958; Fig. 4.1A), has a long history of research, broad spatial and stratigraphic distributions and both shallow- and deep-marine environmental tolerance (Grazhdankin *et al*., 2008; Droser and Gehling 2013; Liu *et al*. 2015). New populations of *C. masoni* offer excellent opportunities to test claims of animal ancestry in Ediacaran rangeomorphs.

I here present a reanalysis of the morphology of *Charnia masoni* and identify features that lead me to propose a new model for its anatomy. This model has significant implications for

our understanding of rangeomorph intra-specific variation, and consequently for rangeomorph taxonomic schemes. The following redescription is undertaken in the expectation that a detailed understanding of anatomy must necessarily precede understanding of an organism's place in phylogeny and, consequently, its evolutionary significance.

## 4.2 Previous work:

*Charnia masoni* is a uniterminal rangeomorph, which is known to range in length from ~1–66 cm (Fig. 4.1; Boynton and Ford 1995; Hoffman *et al.* 2008; Liu *et al.* 2012). It comprises a holdfast, stem and tapering ovate to parallel-sided frond (Laflamme *et al.* 2007) consisting of two rows of first order branches (Fig. 4.1A; terminology follows Brasier *et al.* 2012). First order branches are longest in the middle of the frond and smallest at the distal tip (Ford 1958). *C. masoni* is considered to belong to the Charniida (Pflug 1970; Glaessner 1979b); a sub-group of Rangeomorpha comprising those taxa with single-sided (rotated; Brasier *et al.* 2012) first order branches (Narbonne *et al.* 2009). The angle of repose of *Charnia* first order branches varies between specimens (both within and across bedding planes) but the form of the organism remains constrained (Dunn *et al.* 2017). First order branches meet in an alternating arrangement at the midline to form a zigzag apico-basal axis, with no visible stalk (Ford 1958; Grazhdankin 2004*a*) and, as such, the growth axis has been considered concealed (Brasier *et al.* 2012). This branch alternation confers glide reflection symmetry (an offset form of bilateral symmetry; e.g. Brasier *et al*. 2012) on the frond. Rarely, groups of first order branches may dislocate from their neighbours (Wilby *et al.* 2015, figs 5–10) but more commonly they present as a tightly stacked arrangement.

First order branches have been described to comprise up to 25 second order branches (Wilby *et al.* 2015), the shape of which may vary from rectangular to sigmoidal along an individual first order branch (Laflamme *et al.* 2007). Second order branches themselves comprise smaller third (Jenkins 1985) and fourth order branches (Brasier and Antcliffe 2009), with each successive branch order oriented broadly perpendicular to the previous one. The branching in *Charnia masoni* has been described as undisplayed and furled at all orders (*sensu* Brasier

*et al.* 2012), with the number of first order branches generally increasing with specimen size (e.g. Antcliffe and Brasier 2008). These observations have led researchers to conclude that *C. masoni* differentiated new first order branches during its life, and that these branches subsequently inflated as the organism grew further (Antcliffe and Brasier 2007, 2008; Wilby *et al.* 2015). New branches have typically been interpreted to differentiate from the apex of the organism (Antcliffe and Brasier 2007), where the smallest first order branches are located, but an additional basal growth zone was proposed in Chapter two, following identification of stems of markedly different relative lengths in some specimens (Fig. 2.3A-B). Whether all four orders of branch division are visible at all observed stages of ontogeny, or whether they emerge during development in a hierarchical fashion (as suggested by Flude and Narbonne 2009), has not yet been resolved.

Although the gross morphology of *Charnia masoni* has been relatively well-characterised, discrepancies exist in the detail to which its component parts have been studied. The morphology of first order branches has been well analysed (e.g. Laflamme *et al.* 2007; Wilby *et al.* 2015), whereas third order and fourth order branches have been little discussed in the literature, presumably due to their small size and incomplete preservation within most specimens. There is therefore ample scope for morphological analysis of these smallest branch divisions using well-preserved specimens.

*Charnia masoni* is widely considered to have been identical on both faces/sides. However, Grazhdankin (2004*a*) has suggested that this may not have been the case and that one face of *C. masoni* possessed characteristic furled and rotated rangeomorph branching architecture at multiple branch orders, whilst the other possessed first and second order branches only. Narbonne *et al.* (2009) describe putative internal anatomy in one specimen termed "*Charnia* cf. *C. masoni*", identifying a possible central stalk with 'tube'-like support structures for the first order and second order branches. They also describe an outer "distal rim" to the frond, which they consider was an internal feature that originally connected to the central stalk and the first order branch support structures (though see Grazhdankin and Seilacher 2005, who interpret 'internal' structures to result from current winnowing, or Brasier *et al.* 2013, who reinterpret both the distal-rim and the internal stalk as sedimentary features related to scouring).

The holdfast of the organism has received little discussion (though see Jenkins 1985; Grazhdankin 2014), possibly because much work has focused on the holotype specimen, where the holdfast has historically been thought to be missing (though see Wilby *et al.* 2015 fig. 5-1). Where present, the holdfast is small and bulbous (Laflamme *et al.* 2007; Wilby *et al.* 2015), though it was described as elongate by Jenkins (1985) and recent work has also suggested that the holdfast of *Charnia masoni* may be more deeply buried than other rangeomorph holdfasts, thus appearing smaller (Burzynski and Narbonne 2015). Occasionally, a stem-like region, sometimes with second order subdivisions (Wilby *et al*. 2015), can be seen in *C. masoni* connecting the holdfast to the frond (the basal extension as defined later). This region is considered distinct from the true, naked stems of other rangeomorphs (Laflamme *et al.* 2012) and non-rangeomorph frondose Ediacaran taxa (e.g. Laflamme *et al.* 2004), which do not possess any second order subdivisions along their stems.

In summary, while *Charnia masoni* is one of the best studied rangeomorph taxa, there remain several crucial aspects of anatomy that are either contentious (e.g. internal anatomical structures), or insufficiently characterized. Some of these are features (e.g. branching architecture) contribute significantly to taxonomic diagnosis in rangeomorphs (Laflamme *et al.* 2008; Brasier *et al.* 2012). Any improvement to our knowledge of *Charnia* anatomy is therefore valuable.

## 4.3 Materials and Methdods:

A total of 47 well-preserved *Charnia masoni* specimens from bed B of North Quarry in the Bradgate Formation, Charnian Supergroup, UK (see Wilby *et al.* 2011), including the holotype (LEIUG 2328, Ford 1958), and 17 specimens from bed LC6 of the Catalina Member of the Trepassey Formation, Newfoundland (see Liu 2016), were studied either in the field or from high resolution casts and molds (housed at the British Geological Survey, Keyworth and the Sedgwick Museum, Cambridge, respectively). Specimens are preserved in low negative epirelief and occupied deep-water turbiditic depositional settings during life (Wood *et al*. 2003). Five additional partial specimens from the Verkhovka Formation, Valdai Group, White Sea region of Russia (Grazhdankin 2004*a*), were analysed from photographs, or at the

Palaeontological Institute (PIN) in Moscow. These Russian specimens are preserved in three dimensions in fine-grained sandstone interbeds alternating with mudstone and representing a storm-influenced middle shoreface depositional environment (Grazhdankin 2004*a*).

Specimens of *Charnia masoni* from Newfoundland were studied either in the field, or from high resolution casts and molds. These were retrodeformed prior to study (a technique used to account for tectonic deformation of specimens; Wood *et al.* 2003) following the constant area method (Heywood 1933) (which represents a minimum estimate of deformation) Specimens from Charnwood Forest were not retrodeformed since all fronds on Bed B are aligned and are considered to have been subjected to the same magnitude of deformation (following Wilby *et al.* 2015). Specimens from the White Sea were not retrodeformed, as the strata are not considered to have undergone significant tectonic deformation (Stankovsky *et al.* 1990; Grazhdankin 2003, 2004*b*) and it is not possible to excavate large enough portions of the bedding plane to obtain suitable independent strain indicators. Due to inherent deformational differences, I do not consider quantitative data derived from these various populations to be directly comparable. However, I do discuss general morphological variation across the different sample areas.

Interpretive illustrations of individual specimens were produced in Adobe Photoshop CC. Silicon molds were made of specimens from Newfoundland in the field, under permits issued by the Government of Newfoundland and Labrador, under Regulation 67/11 of the Historic Resources Act.

While all specimens were examined, measurement data was only collected from a subset of specimens. This was because many specimens of *C. masoni* do not preserve either the apex and/or base of the specimen, or the lateral margins. This makes quantitative data derived from these specimens unreliable.

A complete specimen list can be found in Appendix one.

## 4.4 Results

### 4.4.1 Specimens from Charnwood

The best-preserved and largest specimens of *Charnia masoni* exhibit four (resolvable) orders of branching (Fig. 4.1), while the smallest specimens (~2 cm) lack the resolution required to determine the number of branch orders originally present. The smallest first order branches are located at the distal tip of individual fronds, which are typically ovate in shape and appear well constrained (i.e. lacking first order branches of aberrant length) in all specimens. One specimen appears to show an area of first order branch dislocation (*sensu* Wilby *et al.* 2015), with the angle of repose of first order branches being higher above the dislocated area (towards the distal tip; Fig 4.1F). First order branches are constructed of rectangular second order branches, which are oriented laterally and basally and are themselves constructed of third and fourth order branches. Third order branches, which are oriented apically, can appear displayed and furled (Fig. 4.1C, terminology cf. Brasier *et al*. 2012), undivided, or rotated and furled.

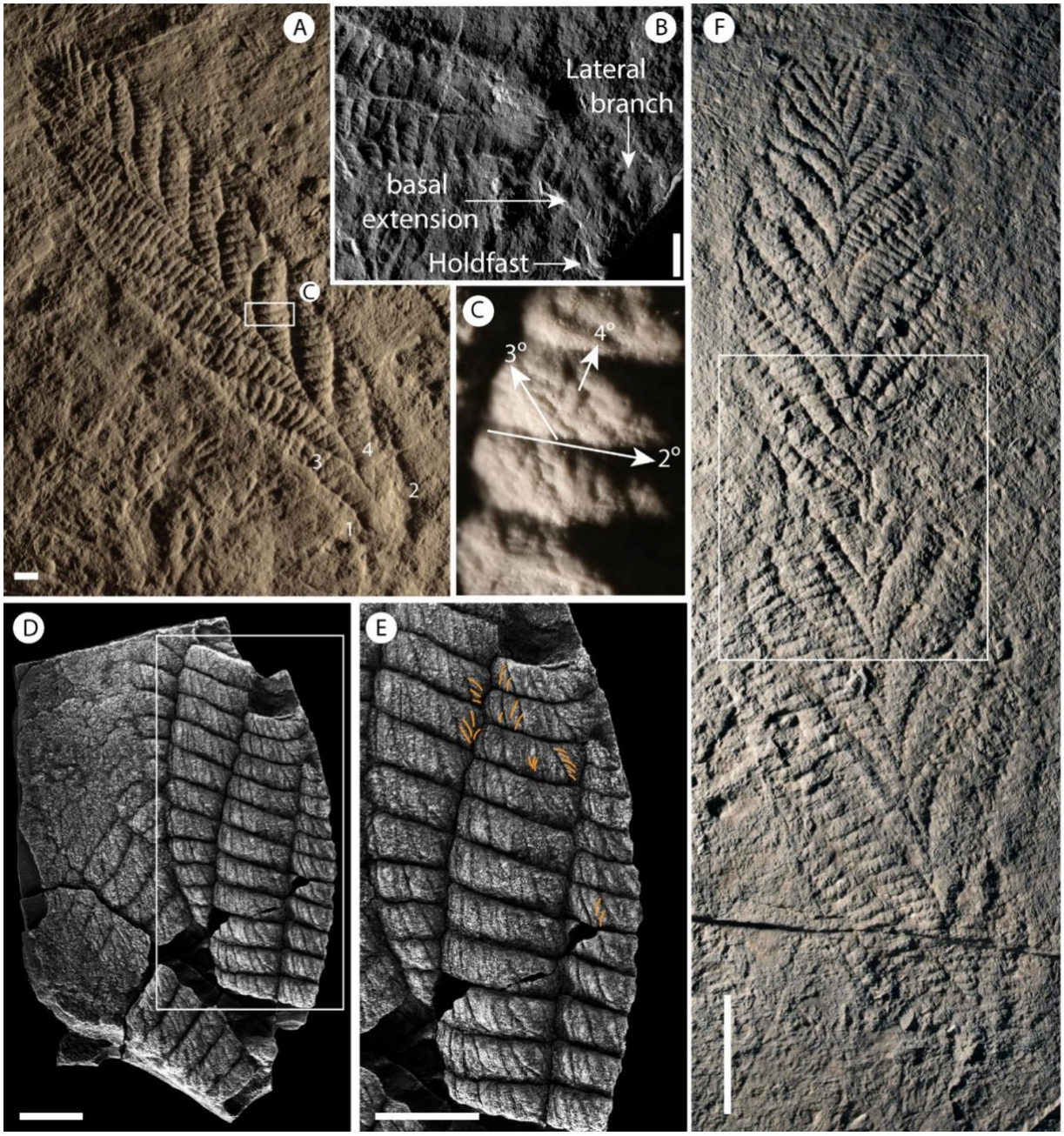


**Figure 4.1: A–C)** *Charnia masoni* Holotype (LEIUG 2328) from Bed B (Wilby *et al.* 2011), North Quarry, Charnwood Forest, UK. **A)** Latex mould of the complete specimen. Lateral branches (the basal-most branch pair) are labelled one and two, branches comprising the basal extension (the next most basal branch pair) are labelled three and four. **B)** Cast of the basal region of the holotype, showing the holdfast, basal extension and lateral branches. **C)** Displayed branch architecture in third and fourth order branches (second order branch marked '2°', third order branch marked '3°' and fourth order branch marked '4°') – holotype mould. **D–E)** Partial *Charnia masoni* specimen from the White Sea (PIN 3993-7018) **E)** High

order rangeomorph branching, examples of rotated or displayed furled fourth order branches are highlighted in orange. **F)** Latex mould of a *Charnia* specimen interpreted to be twisted, Wilby *et al.* (2015) (GSM 105873). The white box highlights the area of inferred twisting. Scale bars **A**–**E** = 10 mm, **F** = 5 cm.

Most first order branches appear to meet in an alternating arrangement in the centre of the organism, conferring a glide symmetrical arrangement. However, the two most proximal branches in individual specimens (closest to the holdfast) do not appear to conform to this pattern, instead connecting directly to the lateral margins of the holdfast (Figs 4.1A–B, 4.2). These two most proximal branches (observed to be present in 8 specimens and absent from 9 specimens, based on the position of their unique attachment point) are morphologically distinct from all other first order branches, with second order branches present along their entire length and third order branches sporadically preserved. I term this pair of first order branches the lateral branches. The next adapical pair of first order branches (i.e. the second pair of first-order branches; Figs 4.1A–B, 4.3) may also appear morphologically distinct, in some cases extending between the most proximal first order branch pair (the lateral branches) to form an area termed the "stem" or stem-like area in Chapter two. This area is variable amongst specimens; it can be present or absent within individuals from a single population (it is present in 9 specimens from Charnwood Forest, out of 19 where the base of the organism is preserved) and it may vary in length within the population (both in absolute and proportional terms; see Table 4.1, Fig. 4.4).

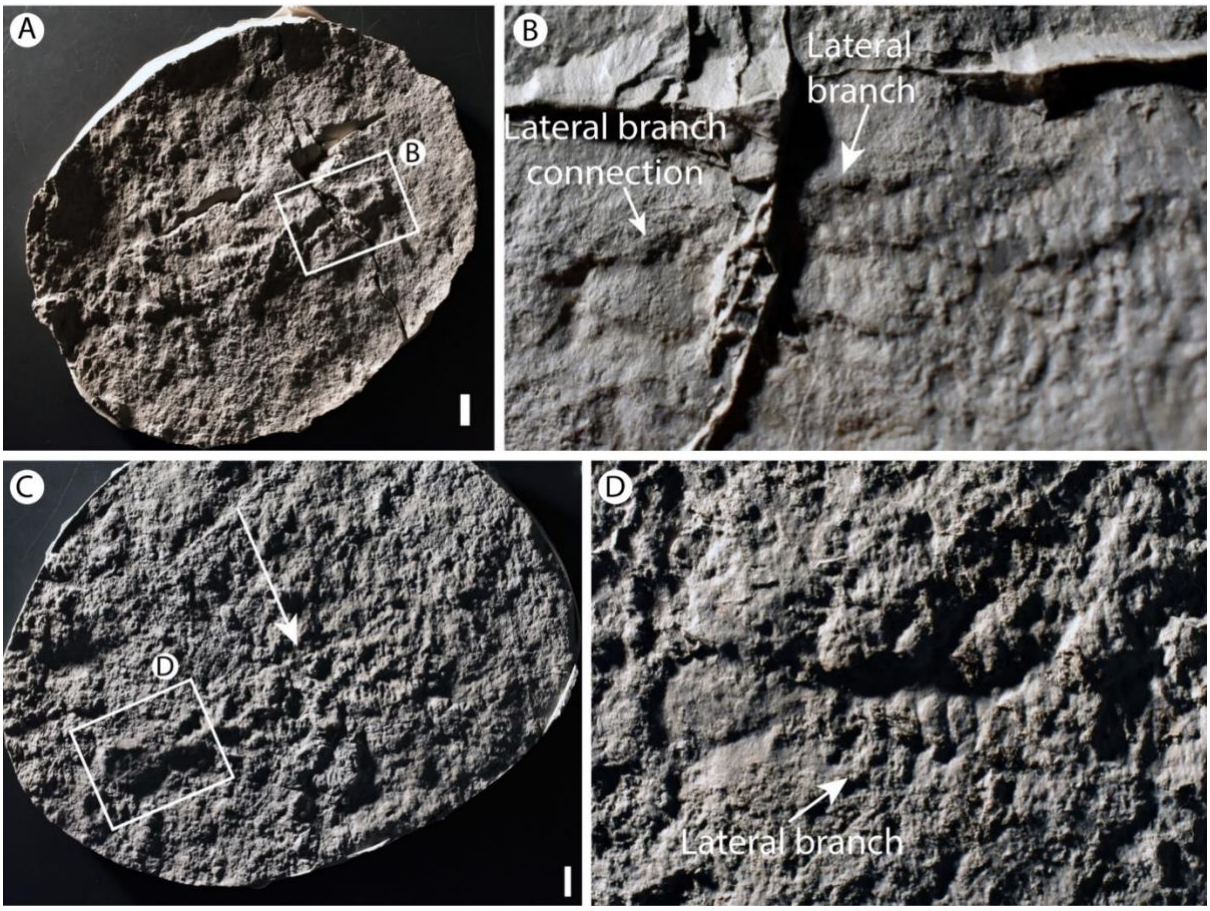


**Figure 4.2:** *Charnia masoni* specimens from Charnwood Forest, UK. **A–B**) Cast of GSM 105993. The arrows in **B)** highlight the basal-most branch as it connects directly to the lateral margin of the holdfast. **C–D)** Cast of GSM 105972. The specimen is arrowed in **C.** In **D,** the arrow points to the basal-most branch, which connects directly to the lateral margin of the holdfast. Scale bars = 10 mm.

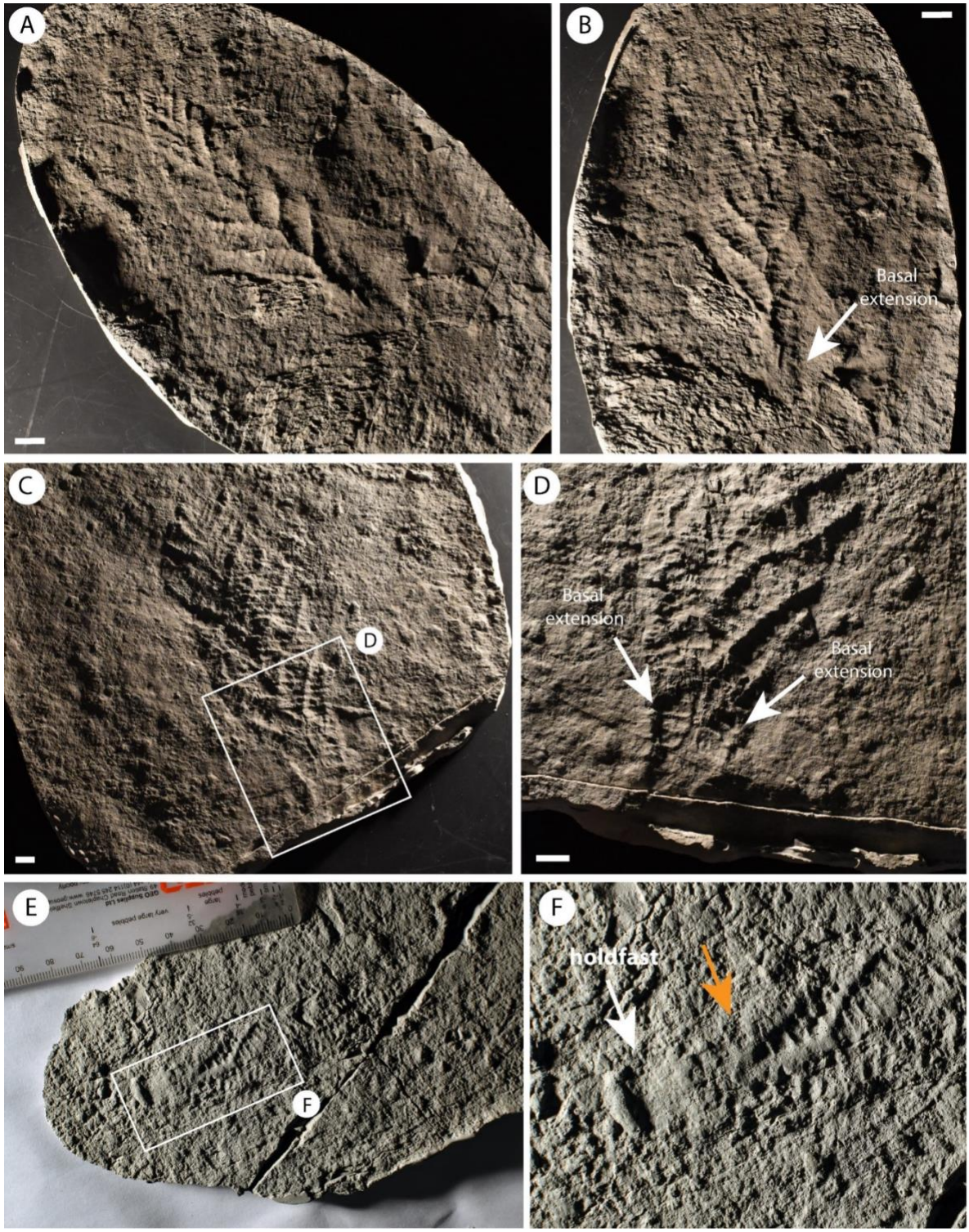


**Figure 4.3:** *Charnia masoni* specimens from Charnwood Forest, UK. **A–B)** Cast of GSM 106078, showing the basal extension. **C–D)** Cast of GSM 105997, showing the basal extension in **D**. This specimen does not preserve a holdfast. **E–F)** Cast of GSM 105966, which does not show a basal extension, but rather the first order branches connect to the holdfast without any expansion near the base of the branch. The holdfast and lowermost branches are arrowed (white and orange arrows respectively) in **F**. Scale bars = 10 mm.

| *Specimen* | *Total Length (mm)* | *Basal extension (mm)* | *Length of the basal extension as a proportion of total organism length* |
|---|---|---|---|
| GSM 105978 | 118 *(4)* | 21 *(1)* | 18% |
| GSM 106040 | >111 *(2.8)* | 12 *(0)* | N/A |
| GSM 105966 | 99 *(0)* | 0 | 0% |
| Holotype | >220 *(2.5)* | 27 *(1.4)* | N/A |
| GSM 106078 | 131 *(2.5)* | 26 *(1)* | 20% |
| GSM 105989 | 77 *(2.5)* | 9 *(1.4)* | 12% |
| GSM 105979 | 101 *(2.8)* | 3 *(0)* | 3% |
| GSM 105997 | >174 *(3.7)* | 27 *(1.4)* | N/A |
| GSM 105972 | 120 *(0)* | 10 *(1.4)* | 8% |
| GSM 106084 | 26 *(1.4)* | 0 | 0% |

**Table 4.1:** Measurements of total specimen length, the length of the basal extension and the relative proportion of the specimen this area comprises. Measurements shown are the mean of three, with standard deviation shown in brackets. "N/A" represents cases where the total length data are not precise, and therefore proportions cannot be accurately determined. Specimens from Bed B, Charnwood Forest, UK.

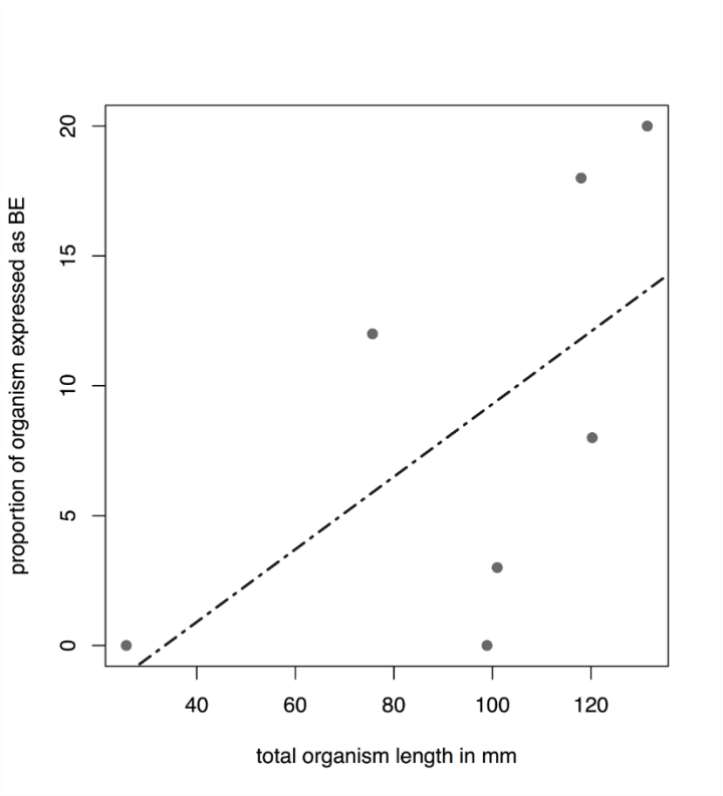


**Figure 4.4:** Data from Table 4.1 plotted in graphical form. The black dashed line represents the best fitting (linear) model, but this is non-significant (P = 0.1483).

A stalk-like structure (n.b. a stalk runs apico-basally through the frond, whereas the stem connects the holdfast to the frond, *sensu* Brasier *et al.* 2012) may be visible near the base of the frond in one specimen (Fig. 4.5A–B) and appears to connect directly to the holdfast. However, similar structures in other specimens appear to be the remains of first order branch boundaries where the branches have been effaced (Fig. 4.5C–D). Such

structures should, therefore, be treated with caution. Where first order branches appear dislocated (Fig. 4.1F), there does not appear to be any suggestion of a central stalk structure.

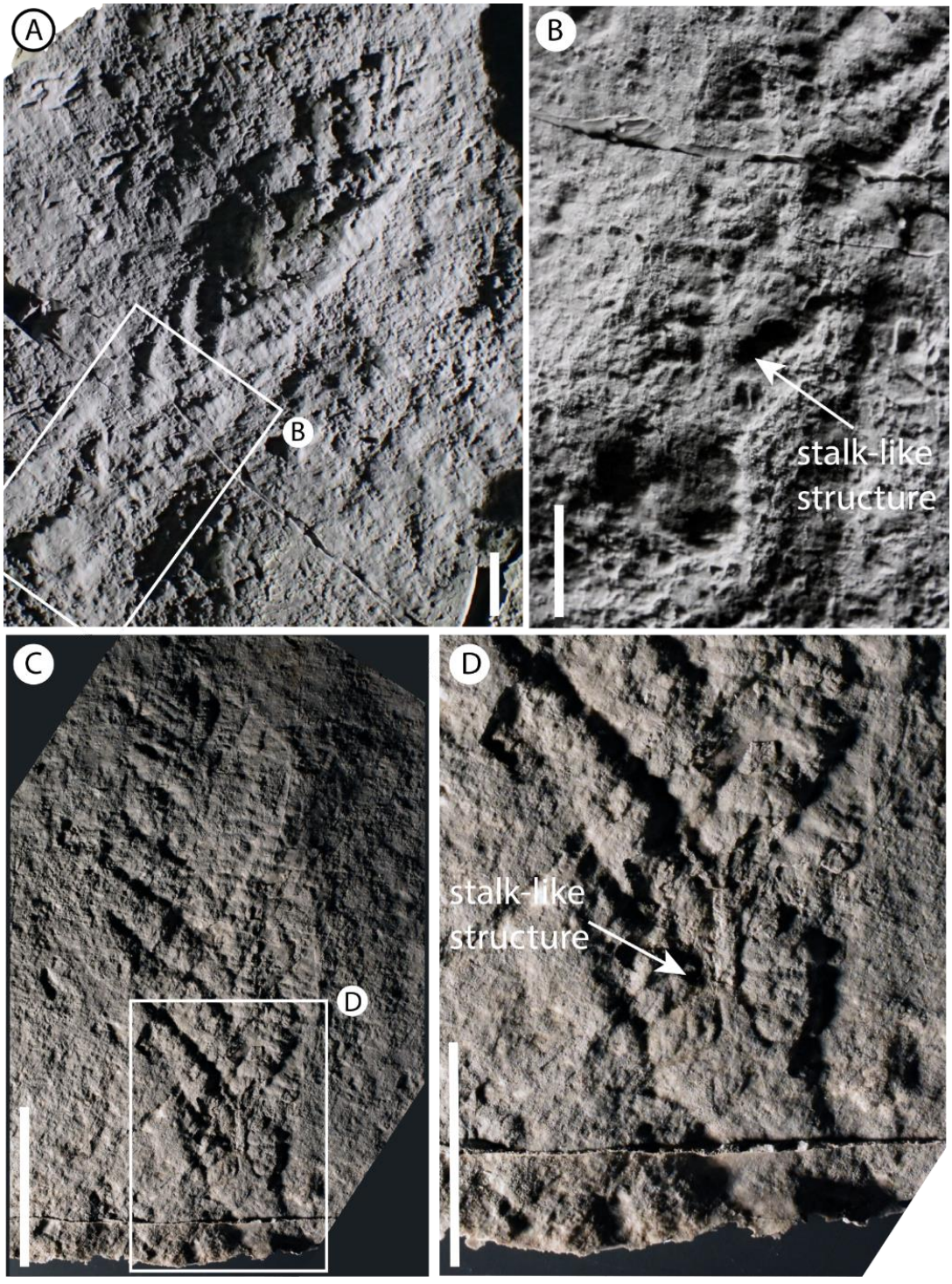


**Figure 4.5: A–B)** *Charnia masoni* specimen cast (GSM 105989), Charnwood Forest, UK. **B)** Base of the specimen in **A**, showing first order branches connecting to a stalk-like structure (arrowed). **C–D)** Mould of specimen GSM 105997, showing what ostensibly appears to be a

stalk-like structure. **D)** The stalk-like region, which appears to represent the effaced remnants of adjacent first order branches. Scale bars = 10 mm.

A holdfast is not observed in the majority of *Charnia masoni* specimens from Charnwood Forest but, where it is observed (16 specimens) it varies from circular to slightly elongate in shape and is generally small (relative to other rangeomorph holdfast structures; e.g. Wilby *et al.* 2011 fig. 2B-C). The possibility remains that it could be potentially buried and therefore not preserved in its entirety on the bedding plane (Burzynski and Narbonne 2015).

### 4.4.2 Specimens from Newfoundland

*Charnia masoni* specimens from Newfoundland include small individuals measuring little over 1 cm in length (Liu *et al.* 2012) and possessing three resolvable orders of branching (Fig. 4.6). Larger specimens may display up to four resolvable orders of branching, with specimens appearing to cluster in two distinct morphs that generally show little/no geographical overlap, but which can co-occur on individual beds. One morph possesses an ovate frond outline, and resembles specimens from Charnwood Forest (e.g. Fig. 4.7E). The other morph exhibits a slender and strongly parallel-sided frond (cf. Laflamme *et al.* 2007; Figs 4.8A–D, 4.9). Both morphs have a constrained frond form, with the smallest first order branches present at the distal tip of the frond and the longest first order branches present in the middle, with first order branches meeting in the centre of the frond in an alternating arrangement. In the parallel-sided morph, which is present on at least five distinct surfaces, second order branches appear sigmoidal in shape, where their lateral margins are preserved. Third order branches may be undivided and furled, or rotated and furled (*sensu* Brasier *et al.* 2012; Fig. 4.8D). Taphonomic constraints prohibit us from drawing conclusions regarding the morphology of the smallest branching orders in the Charnwood-type morph.

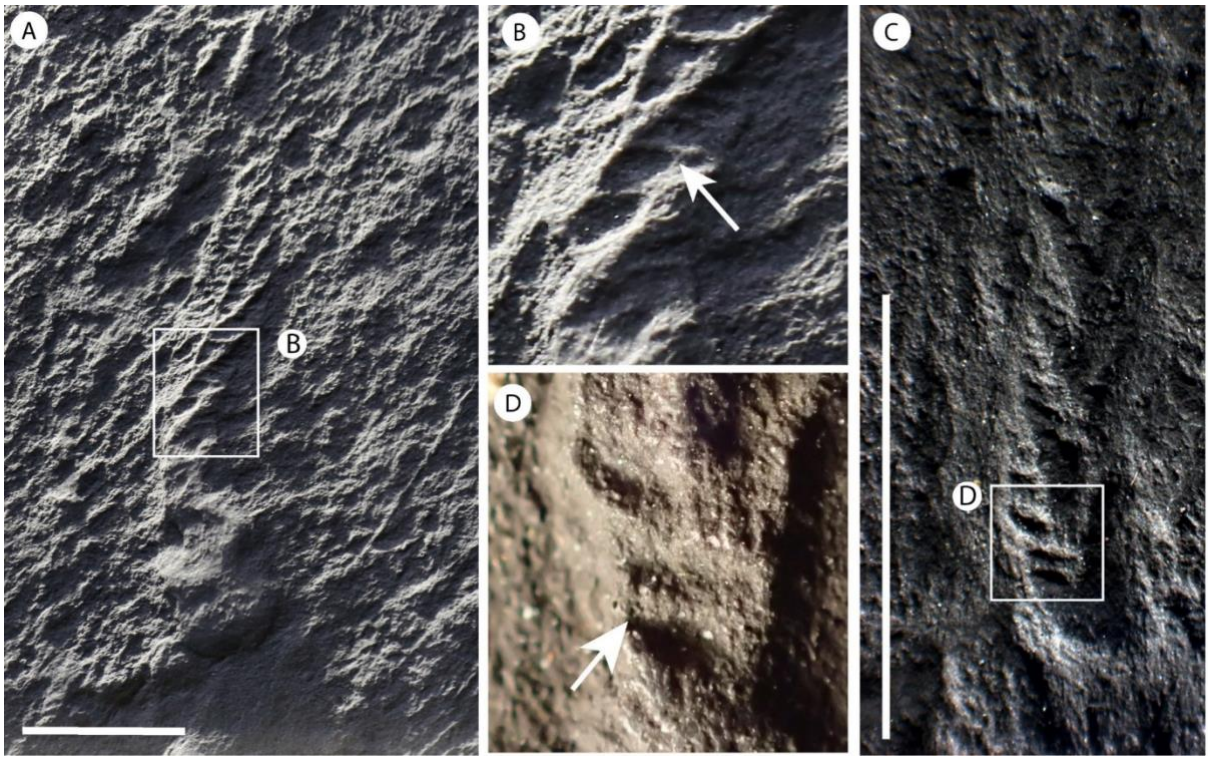


**Figure 4.6: A)** *Charnia masoni* (cast) from the MUN surface, Newfoundland, Canada (Liu *et al*. 2016) (Sedgwick X.50297.9) showing third order branching, highlighted in **B**. **C)** The smallest described specimen of *C. masoni* (specimen OUMNH ÁT.429/p) from Pigeon Cove, Newfoundland, Canada (Liu *et al.* 2012) with third order branching highlighted in **D**. Scale bars = 10 mm.

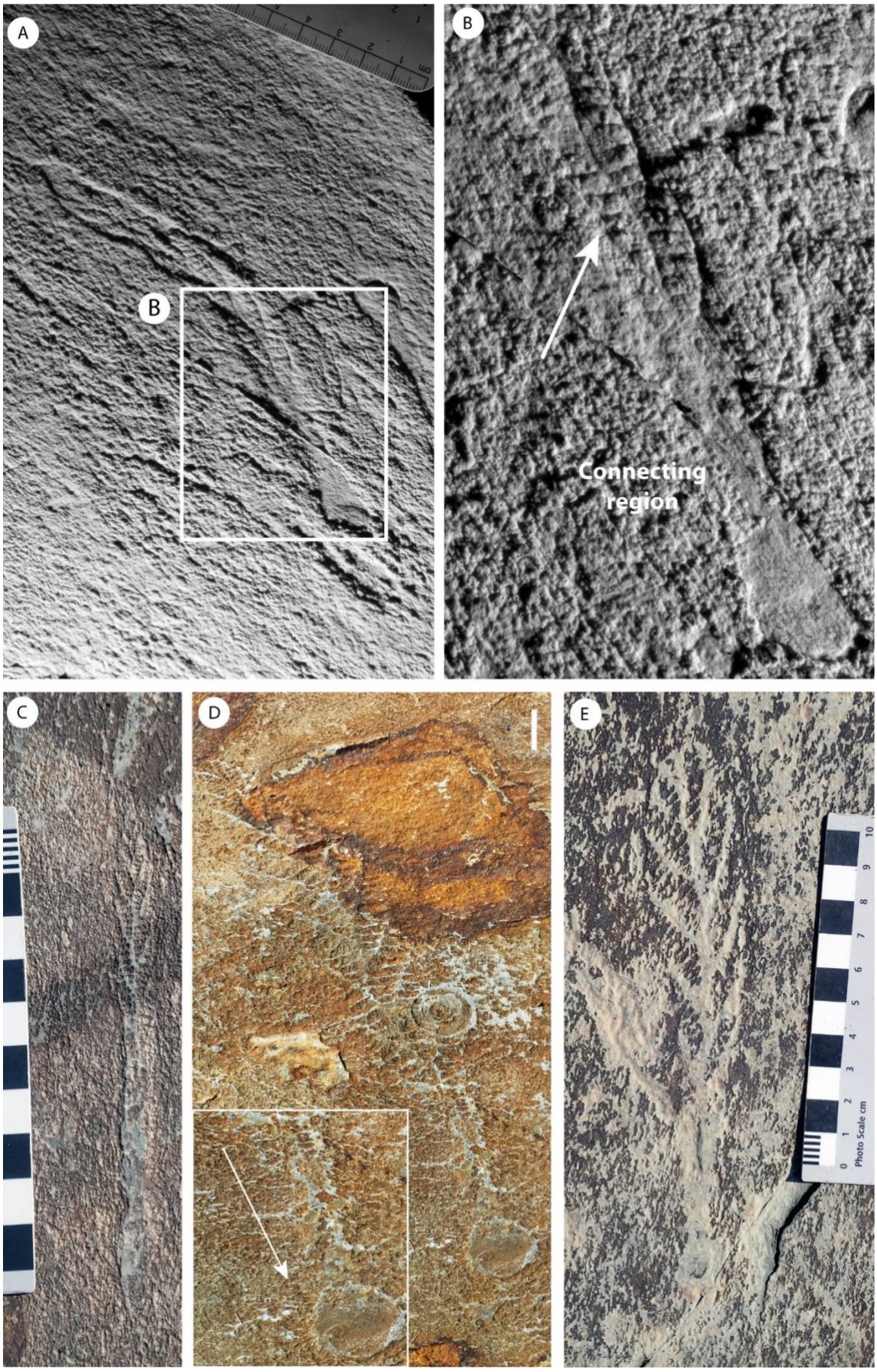
A
B
B
Connecting region
C
D
E
Photo Scale cm

**Figure 4.7:** Specimens of *Charnia masoni* from locality LC6, Bonavista Peninsula, Newfoundland, Canada. **A)** Silicon mould of a slender (parallel-sided) specimen (Sedgwick X. 50297.10) with what I term the 'connecting region', showing sigmoidal first order branching extending much of the way down the specimen, shown in **B**. **C)** Parallel-sided specimen with a connecting region preserved in positive epirelief (cast of specimen, Sedgwick X.50297.2). **D)** Specimen with a basal extension in the connecting region (cast of specimen, Sedgwick X.50297.1). Arrow in the inset shows the branch connections to the holdfast. **E)** Charnwood-like specimen with first order branches showing 'connecting region' typical of parallel-sided specimens from this surface. Images are retrodeformed, except specimen in **C** due to lack of available holdfast structures. Scale bars/gradations = 10 mm.

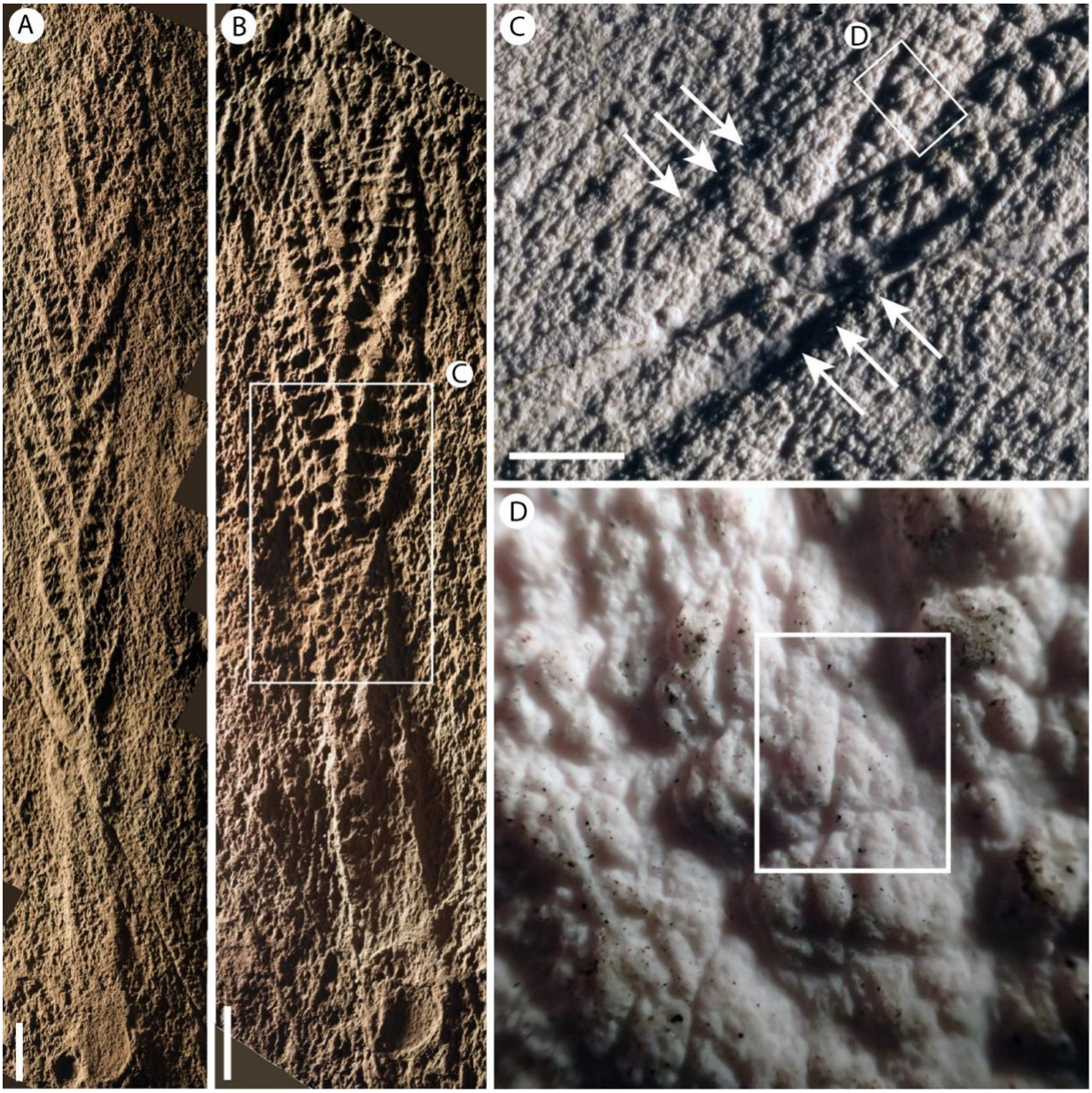


**Figure 4.8:** Specimens of *Charnia masoni* from Newfoundland, Canada. **A–B)** Casts of specimens from bed LC6 (Sedgwick number X.50297.5 and X.50297.4). **C)** The basal area of the specimen in **B**, with second order branches visible (arrowed) on adjacent first order branches running down into the connecting region. **D)** Mold showing rotated and furled third order branches, highlighted by white box, from the specimen in **B**. Images were retrodeformed using the constant area method. Scale bars = 10 mm.

In certain specimens of the parallel-sided morph from two individual bedding planes in Newfoundland (LC6 and Site 40 of Hoffman *et al.* 2008), the frond is connected to the holdfast via a long connecting region that is narrower than the frond (Fig. 4.7–4.8). On both beds, *Charnia masoni* specimens with this connecting region are considerably more abundant than specimens without (no specimens without the connecting region are documented on Site 40, while only two are documented on LC6, in contrast to ~20 specimens that possess a connecting region). This area is commonly preserved in positive epirelief, in contrast to the negative epirelief preservation of the frond branches (Fig. 4.7D). It may display first and second order branching at least part way along its length (Figs 4.8A-C, E, 4.9), with a bias towards preservation of only one row of first order branches (e.g. Fig. 4.7B–C). Within this connecting region, effaced first and second order branching is often visible (e.g. Fig. 4.7–4.8). The length of the connecting region located proximally to the basal-most expression of distinct first or second order branching is variable within populations (Table 4.2, Fig. 4.9) and is not tightly correlated to specimen size. A holdfast is commonly preserved in specimens from Newfoundland, and can exhibit circular to slightly elongate morphologies (Figs 4.8–4.9).

| ***Specimen*** | ***Total length (mm)*** | ***Connecting region (mm)*** | ***Length of connecting region as a proportion of total organism length*** |
|---|---|---|---|
| X. 50297.11 | 89 *(0)* | 15 *(2)* | 17.02% |
| X. 50297.7 | 103 *(2.2)* | 23 *(2)* | 23.63% |
| X. 50297.1 | 141 *(0)* | 0 | N/A |
| X. 50297.10 | 147 *(1.4)* | 16 *(2.4)* | 10.71% |
| X. 50297.4 | 243 *(3.7)* | 34 *(1.4)* | 13.9% |
| X. 50297.5 | 158 *(2.4)* | 34 *(0)* | 21.34% |

**Table 4.2:** Comparison of specimen total length and connecting region length in specimens from locality LC6, Newfoundland, Canada. Measurements were taken from casts of specimens. Measurements shown are the mean of three, with standard deviation shown in brackets. Only specimens where the base of the organism is well preserved were included in my analysis. “N/A” represents cases where the total length data are imprecise, and therefore cannot be used to accurately determine proportions.

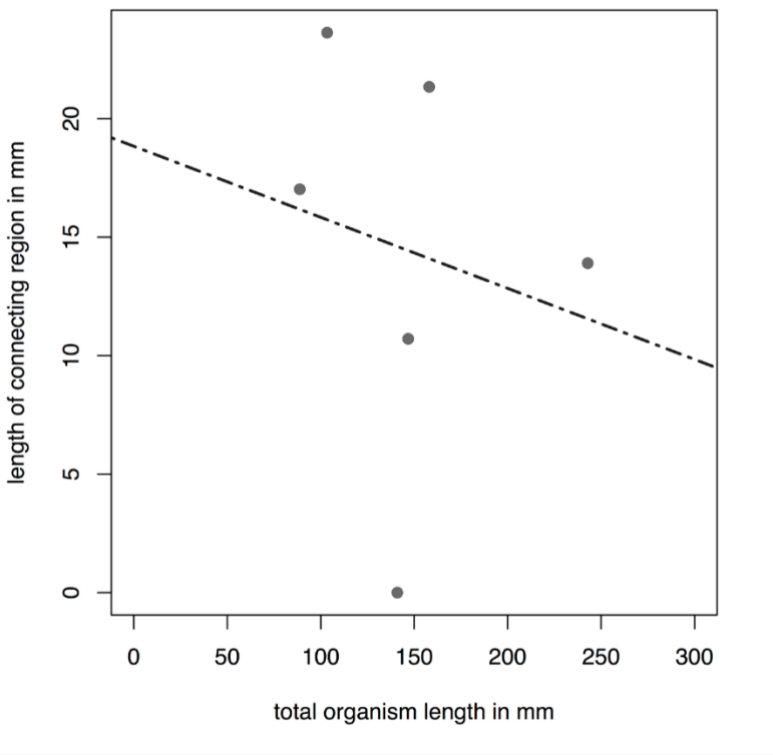


**Figure 4.9:** Data from Table 4.2 plotted in graphical form. The black dashed line represents the best fitting (linear) model, but this is non-significant (P = 0.7174). Images were retrodeformed prior to measurement using the constant area method.

### 4.4.3 Specimens from Russia

All examined specimens from the White Sea are incomplete and so no comments about gross form can be made. Four orders of branching were noted in well preserved areas (Fig. 4.10D–E), and first order branch form appears constrained. First order branches meet along the midline in an alternating fashion, conferring glide symmetry upon the frond. The exposed area in Figure 4.1D–E highlights the tight packing of first order branches. I find no evidence for a central stalk in this exposed area, or in any of the Russian specimens. As with specimens from Newfoundland, second order branches may be rectangular or sigmoidal (furled or displayed; Fig. 4.10D). Where second order branches are disarticulated (e.g. Fig. 4.10D), the boundary between these branches appears clean. Third order branches may appear either furled and undivided (Fig. 4.10A–B), rotated and furled, or displayed and furled (Figs 4.1–E, 4.12E). As with specimens from Newfoundland, the basal margins of third order branches (across one second order branch) are more evenly spaced than the apical margins, which appear to be oriented medially in many cases (e.g. Fig. 4.10A–B), suggesting that the third order branches attach to a support structure located basally in each second order branch.

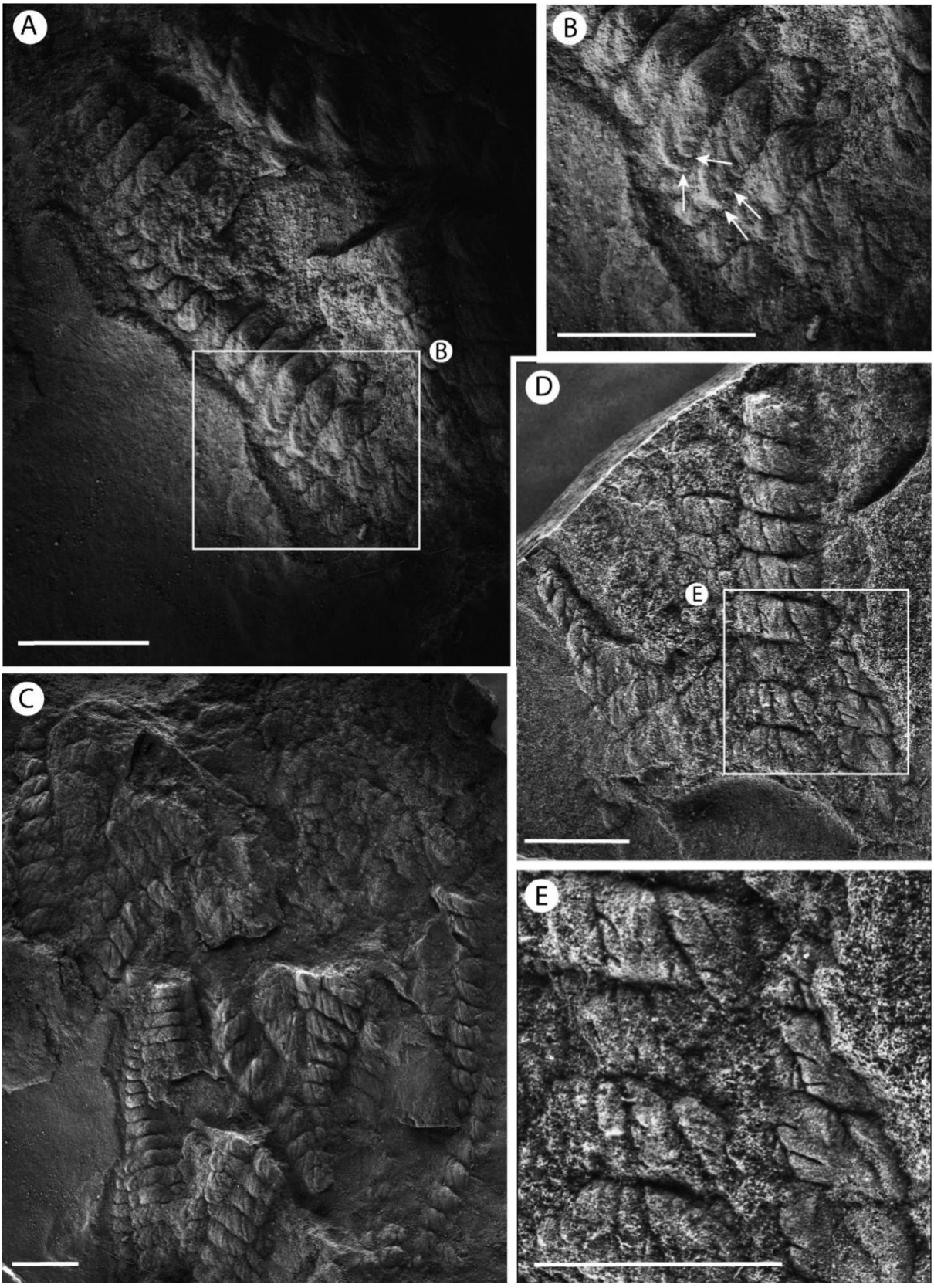
A
B
B
C
D
E
E

**Figure 4.10:** *Charnia masoni* from the Winter Coast of the White Sea, Russia. **A**–**B)** (PIN 3993-7023) Rotated and furled third order branches evenly spaced at the base of a second order branch but oriented medially at the apex. **C)** (PIN 3993-7023) Clean separations between second order branches, and variation in their width of separation, indicate that the second order branches were probably discrete units each with its own boundary wall (rather than a shared wall with adjacent second order branches). **D)** (PIN 3993-7025) Inflated fourth order branches (expanded in E) with no further subdivisions visible. Scale bars = 10 mm. Images kindly provided by D. Grazhdankin.

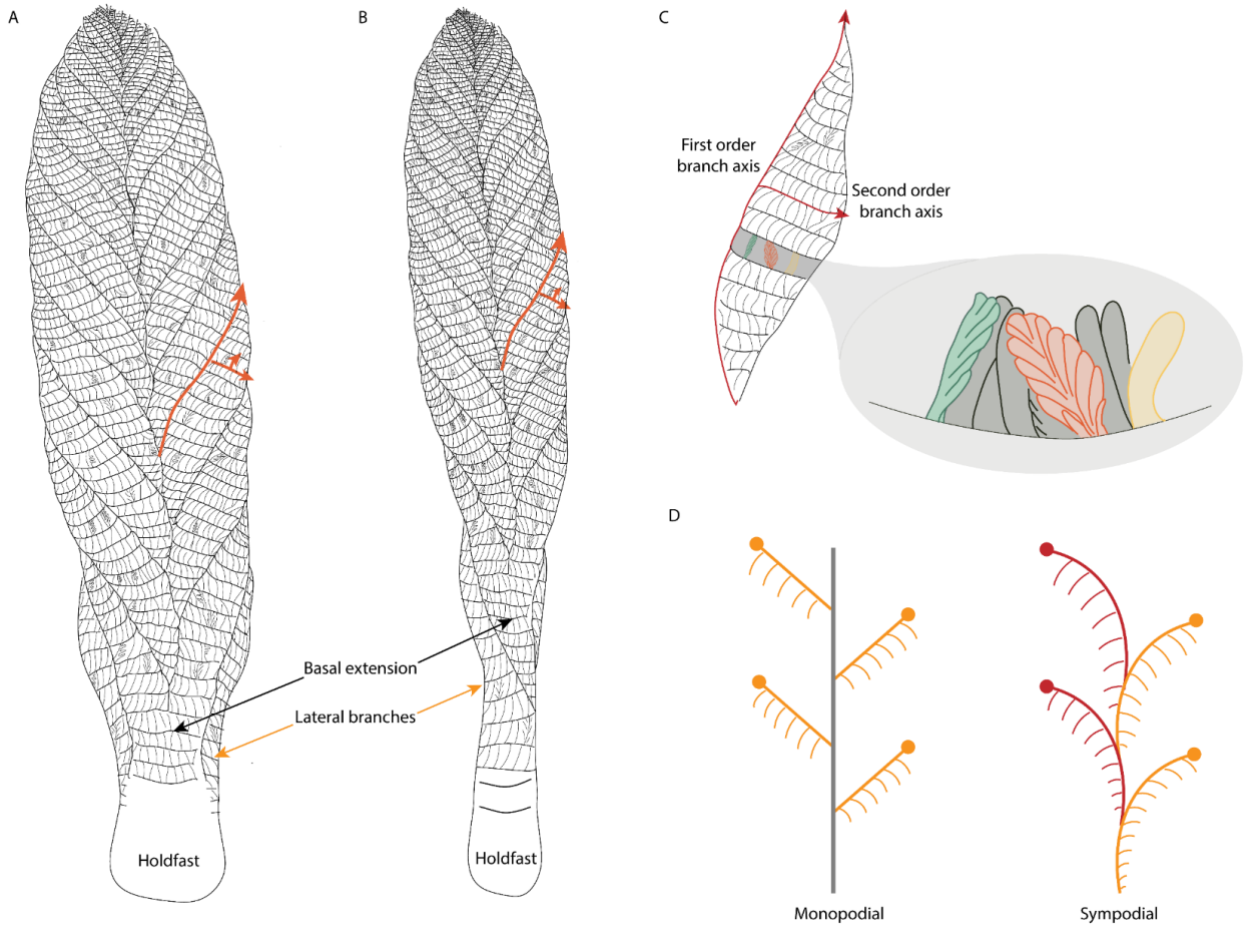


**Figure 4.11:** Morphological model of *Charnia masoni*. **A–B)** Charnwood-like and parallel-sided morphotypes of *Charnia masoni*, respectively. Orange arrows indicate the orientation of the branch axis up to third order. Rotation around central axis is illustrated in **B**. **C)** Observed variation in third and fourth order branch organisation. The orange branch is displayed and unfurled (see Fig. 1C), the green branch is rotated and unfurled (see Fig. 3G) and the yellow branch is undivided and furled (see Fig 6E). Terminology after Brasier *et al.* (2012). Red arrows indicate the first order branch axis (oriented apically) and the second order branch axis (oriented laterally) **D)** Monopodial and sympodial central axial arrangements. Monopodial growth is characterised by lateral branches emerging from a single central axis, while sympodial growth is characterised by successively stacked lateral branches, without a separate central axial structure (e.g. Berking 2006).

## 4.5 Discussion

Integration of the information above allows construction of a new morphological model that better reflects the anatomy of *Charnia masoni* (Fig. 4.11). In the following section, I first discuss the frond and then move basally down the organism to the holdfast.

First order branches in *Charnia masoni* were already known to (rarely) dislocate from each other (Wilby *et al.* 2015), suggesting the presence of only a weak connection between adjacent branches or, alternatively, a stacked arrangement of non-conjoined branches (bound together only at the central axis, or alternatively attached to an axis independent of each other). Evidence indicating that the basal margin of one first order branch could overlie the apical margin of the previous first order branch (Grazhdankin 2004*a*, fig 2D; Laflamme *et al.* 2007) perhaps supports the latter hypothesis. I do not find evidence for a marginal rim (*sensu* Narbonne *et al.* 2009), or any other connective structure inferred to surround first order branches. *Charnia masoni* possesses three further orders of branch subdivision (totalling four orders of branching). It is not currently possible to determine whether the observation that only three branching orders are visible in the smallest, presumed youngest, specimens results from ontogenetic, or taphonomic, processes.

First order branches are sigmoidal in shape and are constructed of second order branches that are rectangular to sigmoidal. Variation in second order branch morphology is the result of the degree of physical rotation each branch has undergone, with fully exposed branches appearing sigmoidal (e.g. Fig. 4.6; see also Laflamme *et al.* 2007), whereas rectangular second order branches appear to have been furled. Second order branches probably possessed their own boundary walls and so it is unlikely they were joined to each other in life along their entire medial-distal axis; they have been joined only at their medial margin. I therefore term this medial margin the first-order branch axis (Fig. 4.11C).

I see no evidence to suggest that first or second order branches in *Charnia masoni* could exhibit a displayed rangeomorph branching architecture in any examined specimens, consistent with previous suggestions of single-sided ‘Charniid’ branching at these branch orders (Narbonne *et al.* 2009; see also thin section data in Grazhdankin 2004*a*, fig 2d.)

While the majority of third order branches appear to conform to the typical furled, rotated or undivided, rotated pattern that defines the genus (e.g. Brasier *et al*. 2012, Wilby *et al.* 2015), individual branches at these higher orders may be furled and displayed, while some are unfurled and displayed (Figs 4.1C, 4.8G). Given the apical orientation of displayed third order branches in specimens from Charnwood Forest, as well as the apical margins of third order branches in specimens from Russia being oriented medially (thus suggesting they were not bound at this margin), third order branches are interpreted to branch apically from their host second order branch along a second order branch axis (Fig. 4.11C). Third order branches also exhibit moderate inflation (*sensu* Brasier *et al.* 2012). Given the rotational variation I observe in fourth order branching, I consider it unlikely that third order branches were conjoined.

Fourth order branches are never observed to show further hierarchical subdivision. I acknowledge that taphonomic constraints may preclude visualisation of further branch orders but note that space constraints do not appear to limit the number of orders visible (e.g. Fig. 4.10E). Fourth order branches typically appear furled and may exhibit moderate (Fig. 4.1C) or medial (Fig. 4.10E) inflation. This is unlike the apparently conserved proximal inflation inferred for first order branches but similar to the moderate–medial inflation inferred for second order branches (Brasier *et al.* 2012).

These observations help to resolve the long-standing question regarding whether rotated (cf. Brasier *et al*. 2012) or "charniid" branches (cf. Narbonne *et al.* 2009) have one or two rows. These specimens (from Charnwood, UK and The White Sea, Russia) demonstrate that rotated branches could be two-sided at higher branch orders, with one side rotated out of the plane of preservation (*sensu* Fig. 4.11C). The potential for (at least third order) rotated branches to appear displayed (Fig. 4.10D–E), and furled branches to appear unfurled (Fig. 4.1C), suggests branching characters at higher (third and fourth) orders are not taxonomically conserved (cf. Kenchington and Wilby 2017). The rotation of these branches supports the notion that at least fourth order branches, and perhaps third order branches in *Charnia masoni*, were not conjoined, but free to move and rotate in the axial plane (cf. Wilby *et al*. 2015).

Branching architecture has significant bearing on the debate surrounding whether *Charnia masoni* had distinct front-back differentiation (see also Grazhdankin 2004*a*). I have been unable to corroborate the identification of two different faces to *C. masoni* in the ~70 specimens directly studied here and therefore infer that both sides of the organism likely possessed the same morphology (see also a Charnwood specimen inferred to be twisted [*sensu* Wilby *et al.* 2015], but display the same morphology above and below the twist, Fig. 4.1F). The apparent absence of third and fourth order branching in some specimens from the White Sea (Grazhdankin 2004*a* fig. 2A) may then represent a taphonomic artefact. The considerable morphological variation in third and fourth order branches (as opposed to first and second order) may suggest that these finer orders of branching played a greater role in nutrient acquisition, as they were free to rotate around their axis. However, this greater flexibility could also simply be a function of their small size and not necessitate functional significance. The lack of evidence for rangiid style branching in the first and second order branches may further suggest that *C. masoni* is not self-similar at every branch order (e.g. Narbonne 2004), although additional evidence is required to confirm or refute this. If this suggestion is borne out, this would undermine the current definition of Rangeomorpha, which requires orders of branching that are identical to "at least three orders" (Erwin *et al.* 2011).

The lateral branches (Fig. 4.2) are morphologically distinct in terms of their unique attachment point to the holdfast, perhaps indicating a greater level of axial complexity to *Charnia masoni* than has previously been inferred (e.g. Hoyal Cuthill and Conway Morris 2014). The next most proximal pair of first order branches may also be morphologically distinct, in some cases extending between the two most proximal first order branches to form an area previously termed the 'stem' (see Chapter two). However, because this area, where present, comprises two individual first order branches rather than a central fused region, I term this area the 'basal extension' (Figs 4.1A–B, 4.12). The basal extension displays some similarity to the proximal section of the subdivided 'axial stalk' (a stem as defined by Brasier *et al.* 2012) described in *Rangea schneiderhoehni* (Vickers-Rich *et al.* 2013). However, in *R. schneiderhoehni* this area is considered a single structure (i.e. not constructed of abutting first order branches). The basal extension is also distinct from the 'naked' stems of other rangeomorphs (e.g. Laflamme *et al.* 2012).

The parallel-sided morph of *Charnia masoni* from Newfoundland possesses a connecting region (Fig. 4.7–4.8), although exact structural reconstruction of this region is hampered by variable quality of preservation near the base of the frond (resulting in often gradational boundaries between the branched area and 'naked' connecting region). This gradational zone, with what appear to be first order branches continuing down the 'connecting region' in many specimens (e.g. Figs 4.8A–B, 4.9A) suggests that this area does not represent a sheath structure (Narbonne *et al.* 2009; though see Brasier *et al.* 2013). This structure could alternatively be interpreted as an artefact of dragging upon felling. However, the presence of first and second order branches that are both aligned with and fit the size profile of other branches in the frond renders this interpretation unlikely. Laflamme *et al.* (2007) document the parallel-sided morph from Lower Mistaken Point on the Avalon Peninsula, but do not describe any form of connecting region, with branches connecting directly to the holdfast (their fig. 6I-J) providing further support that this area may not be a 'stem'. Taken together with the variability in presence and appearance of branches in the connecting region in bedding plane populations of specimens in Newfoundland, the connecting region likely represents an artefact of specimen twisting upon felling and burial. Twisting would not necessarily affect branch preservation in more apical regions, but could result in the apparent absence or poor preservational fidelity of branches closer to the base of the frond.

The base of the *Charnia masoni* frond thus appears to reflect an area with considerable morphological variation, perhaps resulting from taphonomic, environmental, and/or biological factors. The proportional length of this region is variable even across specimens of a similar size from the same bedding plane (Table 4.1, Fig. 4.4). Some of this intra-specific variation may suggest a hitherto unrecognised plastic element to *C. masoni* growth and morphology, and a potential capacity to respond to local environmental factors (e.g. neighbour competition or nutrient availability) by differential morphometric growth (cf. Kenchington and Wilby 2017; Hoyal Cuthill and Conway Morris 2017).

None of the examined specimens show evidence for an internal stalk running along the length of the organism, such as that seen in other rangeomorphs (e.g. *Avalofractus abaculus*, Narbonne *et al.* 2009, or *Rangea schneiderhoehni*, Vickers-Rich *et al.* 2013; Sharp *et al.* 2017). Stalk-like structures observed in my investigations are interpreted as the effaced remains of

first order branch margins (Fig. 4.2C–D). Indeed, space constraints (highlighted by Grazhdankin, 2004*a*) may mean that the presence of such a stalk in *Charnia masoni* is unlikely. An alternative scenario involves the central axis in *C. masoni* being constructed by successively stacked lateral branches (schematically represented in Fig. 4.11D), conferring a sympodially organised central axis (as opposed to a monopodial arrangement present in *Avalofractus* or *Rangea*). This problem is addressed in detail in Chapter 5. I note here the distinctive nature of the basal-most branches in *C. masoni*, which differentiate directly from the holdfast (Dunn *et al.* 2017).

Previous taxonomic schemes for rangeomorphs have placed emphasis on an internal stalk (Laflamme and Narbonne 2008a; Brasier *et al.* 2012) and whether it is exposed or concealed. Narbonne *et al.* (2009) illustrate a structure they interpret as an internal stalk in a *Charnia*-like frond. However, this structure could alternatively be explained as artefact (in appearing to lie too external, in lying atop first order branches, to be the remnants of an internal stalk [Brasier *et al.* 2013]) and, given the very small number of such known examples, I do not consider it a compelling morphological feature. Stalks (as opposed to stems) are assumed but not demonstrated to be present in several other rangeomorphs including the uniterminal *Beothukis mistakensis* and *Beothukis plumosa*, or the biterminal rangeomorph genus *Fractofusus.* Some extant frondose organisms (e.g. hydrozoan cnidarians) are known to display intra-clade variation in axial arrangement (e.g. Berking 2006) and so the assumption that all rangeomorphs must share similar axial arrangements may be erroneous.

The morphology of the holdfasts in *Charnia masoni* can vary markedly between different specimens (Grazhdankin *et al.* 2008, fig. 2A; Wilby *et al.* 2015, fig. 4), ranging from circular to diamond in shape. This variation could represent either true biological or taphonomic (Burzynski *et al.* 2017) variation, or a combination of the two. Perhaps the simplest scenario is that differing depths of holdfast burial account for the majority of observed variation in our studied populations.

The redescription of *Charnia masoni* allows construction of a new model for its *in vivo* anatomy (Fig. 4.11). The organism was attached to the sediment by a bulbous holdfast and was constructed of a series of stacked first order branches arranged in two rows, which may

have been derived successively from a sympodial central axis, or from a cryptic monopodial axis. Each first order branch had an apical axis from which a series of second order branches emerged laterally. Third order branches were attached to the second order branch axes and were oriented apically. Variation in both original anatomy and in preservation near the base of the organism results in the variable presence or absence of both a basal extension, and the lateral branches, in fossilised specimens.

## 4.7 Systematic Palaeontology

Genus CHARNIA Ford 1958

*Emended diagnosis*: Frond uniterminal, comprising two rows of non-conjoined first order branches arranged alternately along a central axis, presenting as a zig-zag medial suture. First order branches typically show proximal inflation, whereas (non-conjoined) second-order units show moderate-to-medial inflation. All first to fourth order branches are aligned in subparallel series. Second order branches are oriented basally, whereas first and third order branches are oriented apically. First order branches comprise rangeomorph elements that are rotated and undisplayed, while second order branches are comprised of rangeomorph elements that may be rotated and either furled or unfurled. There is variation in the presentation of third and fourth order rangeomorph branch elements, which can be displayed and unfurled, displayed and furled, undisplayed and furled, or undivided. A basal disc is sometimes present.

Type species: *Charnia masoni* Ford 1958

v* 1958 *Charnia masoni* Ford 1958, p. 212, pl. 13, fig. 1.

? 1959 *Charnia* sp.; Glaessner, p. 1472, text-fig. 1b.

? 1959 *Rangea?*; Glaessner, *in* Glaessner and Daily, p. 387, pl 46, fig. 2.

1961 *Charnia* sp.; Glaessner, p. 75, text-fig.

1962 *Charnia* sp.; Glaessner, p. 484-485, pl. 1, fig 4 (non fig. 5).

1962 *Charnia masoni*; Ford, fig. 4 (non fig. 5).

1966 *Rangea grandis*; Glaessner and Wade, p. 616, pl. 100, fig. 5.

1972a *Rangea sibirica*; Solokov, pl. I, fig. 3.

1972b *Rangea sibirica*; Solokov, p. 50

1973 *Glaessnerina grandis*; Germs, p. 5, fig. 1D.

1976 *Charnia* ex gr. *masoni*; Sokolov, p. 141

1977 *Charnia* ex gr. *masoni*; Sokolov, p. 441

1978 *Charnia masoni*; Fedonkin, fig. 3 (9).

1979 *Charnia masoni*; Glaessner, fig. 12 (3).

1979 *Glaessnerina sibirica*; Glaessner, fig. 12 (1)

1981a *Charnia masoni*; Fedonkin, p. 66, pl. 3, figs 5, 6; pl. 29, fig 1.

1981a *Zolotytsia biserialis*; Fedonkin, p. 67–68, pl. 3, fig. 7.

1981b *Charnia masoni*; Fedonkin, p. 100.

1981 *Charnia masoni*; Sokolov and Brekhovskikh, p. 3.

1981 *Glaessnerina grandis*; Glaessner and Walter, fig. 6.11 (C).

1983a *Charnia masoni*; Fedonkin, fig. 37.

1983b *Charnia masoni*; Fedonkin, pl. 1, fig. 1.

1983 *Charnia masoni*; Sokolov and Fedonkin, p. 13, fig. 9.

1984 *Charnia masoni*; Sokolov, p. 6, fig. 1.

1984 *Charnia masoni*; Glaessner, fig. 2.21 (A).

1984 *Glaessnerina sibirica*; Glaessner, fig. 2.21 (D).

1984 *Glaessnerina grandis*; Glaessner, fig. 2.21 (C).

1984 *Charnia masoni*; Sokolov and Fedonkin, 1984, fig. 3 (f).

1984 *Charnia* cf. *C. masoni*; Glaessner, fig. 2.21 (B)

1985 *Charnia masoni*; Fedonkin, p. 99, pl. 12, fig 4; pl. 13, figs 2-4

1985 *Charnia* cf. *C. masoni*; Jenkins, fig. 7 (C).

1985 *Charnia masoni*; Jenkins, fig. 7 (B).

1987 *Charnia masoni*; Fedonkin, pl. 15.

1987 *Glaessnerina grandis*; Preiss, p. 310, fig. E.

1990 *Charnia masoni*; Fedonkin, fig. 1(D).

1992 *Charnia masoni*; Fedonkin, fig. 28–30.

1992 *Charnia masoni*; Runnegar, Fedonkin, fig. 7.5.5 (A), fig. 7.5.10 (A)

1994 *Charnia masoni*; Fedonkin, fig. 2 (A, B).

v*1995 *Charnia grandis*; Boynton and Ford, p. 168, fig 1.

1996 *Glaessnerina grandis*; Jenkins, p. 35, fig. 4.1

v1997 *Charnia masoni*; Grazhdankin and Bronnikov, p. 794, fig. 2 (a, d).

?1998 *Charnia masoni*; Nedin and Jenkins, p. 315, fig. 1.

1999 *Charnia grandis*; Ford, p. 231, fig. 3.

v2000 *Charnia*; Martin, Grazhdankin, Bowring, Evans, Fedonkin and Kirschvink, fig. 4 (A).

2001 *Charnia masoni*; Narbonne, Dalrymple and Gehling, p. 32, pl. 1C

v2004 *Charnia*; Grazhdankin, p. 207, fig. 2.

2005 *Charnia masoni*; Narbonne, Dalrymple, Laflamme, Gehling and Boyce, p. 28, pl. 1L.

v2005 *Charnia*; Grazhdankin, Maslov, Mustill, Krupenin, fig. 3 (d).

v2007 *Charnia masoni*; Laflamme, Narbonne, Greentree and Anderson, p. 243, fig. 4A-J

v2007 *Charnia* sp.; Fedonkin, Gehling, Grey, Narbonne and Vickers-Rich, p. 128, fig. 232 *(partim)*

v2007 *Charnia* cf. *masoni*; Fedonkin, Gehling, Grey, Narbonne and Vickers-Rich, p. 145, fig. 276 *(partim)*

v2007 *Charnia* cf. *masoni*; Fedonkin, Gehling, Grey, Narbonne and Vickers-Rich, p. 160, 165, figs. 304, 314 (*partim)*

v2007 *Charnia masoni*; Fedonkin, Gehling, Grey, Narbonne and Vickers-Rich, p. 186, fig. 354

2008 *Charnia masoni*; Hoffman, O'Brien and King, p. 17 (*partim)*, fig. 13.1

v2008 *Charnia grandis*; Hoffman, O'Brien and King p. 18, fig. 14.

v2008 *Charnia masoni*; Grazhdankin, Balthasar, Nagovitsin and Kochnev, p. 804, fig. 2A.

v2009 *Charnia masoni*; Bamforth and Narbonne, p. 907, fig. 7.5

v2011 *Charnia masoni*; Wilby, Carney and Howe, pp. 656-657 (*partim)*, figs 2A, 3A.

v2011 *Charnia masoni*; Grazhdankin, fig. 3 (a–d).

v2012 *Charnia masoni*; Liu, McIlroy, Matthews, Brasier, p. 397, fig. 4B

v2012 *Charnia masoni*; Liu, McIlroy, Matthews, Brasier, p. 397, fig. 5A

v.2013 *Charnia* aff. *masoni*; Liu, McIlroy, Matthews and Brasier, p. 24, fig. 1D

v2013 *Charnia masoni*; Liu, McIlroy, Matthews and Brasier, p. 24, fig. 2A

v2013 *Charnia masoni*; Liu, McIlroy, Matthews and Brasier, p. 24, fig. 2B

v2013 *Charnia masoni*; Liu, McIlroy, Matthews and Brasier, p. 24, fig. 2C

v2013 *Charnia masoni*; Liu, McIlroy, Matthews and Brasier, p. 24, fig. 2D

.2013 *Charnia* sp.; Gehling and Droser, p. 449, fig. 2Q

V2014 *Charnia*; Narbonne, Laflamme, Trusler, Dalrymple and Greentree, p. 213, fig. 5 1-4

v2014 *Charnia masoni*; Grazhdankin, p. 271 fig. 2.3.

v2015 *Charnia masoni*; Wilby, Kenchington and Wilby, p. 20, fig. 2.1,3,6, fig. 2.2,4, fig. 2,5

v2015 Incomplete frond; Wilby, Kenchington and Wilby, p. 20, fig. 2.8

v2015 *Charnia masoni*; Liu, Kenchington and Mitchell, p. 1361, fig. 2D

v2016 *Charnia masoni*; Liu, Matthews and McIlroy, p. 5 *(partim),* fig. 3D.

v2017 *Charnia masoni*; Antcliffe, Liu, Menon, McIlroy, McLoughlin and Wacey, p. 27, fig. 4E

v2017 *Charnia masoni*; Dunn, Liu and Donoghue, p. 5, fig. 1E

v2017 *Charnia masoni*; Dunn, Liu and Donoghue, p. 7, fig. 3

*Diagnosis*: As per genus

*Remarks:*

I do not consider the described variation between specimens of *Charnia masoni* from Charnwood, Russia and Newfoundland to be taxonomically significant. Following recent taxonomic discussions on rangeomorphs, I consider all studied specimens to at least belong within the same genus on the absence of discrete character differences (cf. Liu *et al.* 2016; Kenchington and Wilby 2017). Determination of whether the specimens represent morphs of the same species, or separate species, is more challenging. Where there is variation in multiple continuous characters within Ediacaran taxa, it has been proposed that this would be sufficient to indicate species level differences (Liu *et al.* 2016), depending on the nature and extent of this variation (Kenchington and Wilby 2017). However, when considering morphs from different localities, it can be extremely difficult to distinguish between interspecific and intraspecific variation (Kenchington and Wilby 2017). Although both parallel-sided (Newfoundland) and ovate (Charnwood, White Sea) morphs of *C. masoni* may be present on individual surfaces (e.g. Fig. 4.7, from bed LC6), such occurrences are rare and there is typically one numerically dominant morph. There is some evidence to suggest that local environmental or ecological factors may affect morphology, with the parallel-sided morph never found in the absence of *Fractofusus*, while the Charnwood-morph is known in the absence of *Fractofusus* and, indeed, all other rangeomorph taxa (e.g. Grazhdankin 2004), although low sample size prevents definitive statements.

If further variation (categorical or continuous *sensu* Kenchington and Wilby 2017) is described in these morphs, I would consider it appropriate to reassess these conclusions. Indeed, if variation in discrete characters is identified, then it may be appropriate to erect a new genus.

## 4.7 Conclusions:

Evaluation of the morphology of *Charnia masoni* from three late Ediacaran assemblages (Charnwood Forest, Newfoundland, and the White Sea) enables assembly of an emended model of morphology for this organism, demonstrating greater levels of intraspecific variation than have previously been documented. *C. masoni* specimens from the different localities are comparable in morphology but show features that cannot easily be reconciled with previous rangeomorph taxonomic regimes, and, potentially, fall outside the current definition of Rangeomorpha. My study reveals that certain characters previously proposed as taxonomically informative, such as the displayed/undisplayed, furled/unfurled nature of branches, are fallible at higher branch orders. I provide an emended diagnosis of *Charnia masoni* to take account of the novel features and variation described herein.

A detailed understanding of anatomy must necessarily precede phylogenetic interpretation, since organisms must be interpreted as the sum of all their parts. This novel interpretation of anatomy in *Charnia masoni* – an organism that is amongst the most widely studied of the Ediacaran Macrobiota – illustrates the potential for obtaining new information from global-scale, population-wide studies of well-preserved Ediacaran specimens.

# Chapter 5:

## The Biology of *Charnia masoni* and the affinities of Rangeomorpha:

**Author contributions:** This study was designed and developed by F.S.D, A.G.L, P.C.J.D. Russian specimens were collected by D.V.G and A.G.L, and access to Russian material was provided by D.V.G. Computed microtomographic data was collected by F.S.D and A.G.L, and synchrotron microtromographic data was acquired by F.S.D and P.C.J.D. Segmentation of tomographic data was conducted by F.S.D and Philip Vixseboxse. F.S.D collected all data pertaining to growth analyses and performed those analyses. F.S.D interpreted the segmented tomographic data. F.S.D collected all data for phylogenetic analysis and performed those analyses. P.R.W conducted the backscatter electron microscopy and elemental mapping. F.S.D produced the first draft of this chapter. F.S.D contributed ~85% of the work presented in this chapter.

# Abstract:

*Charnia masoni* Ford 1958 is one of the oldest representatives of the Ediacaran Macrobiota, recovered from rocks as old as 571 million years. In Chapter two, I demonstrated the animal affinity of rangeomorph fossils, but their precise position remains controversial. I quantitatively assess the growth and morphogenesis of *C. masoni* in a population of specimens from Charnwood Forest, UK, supplemented with specimens from the White Sea of Russia that preserve three-dimensional anatomy. I conclude that the growth of *C. masoni* was highly regulated, and involved temporal and regional specificity. This is in contrast to previous models of rangeomorph growth, which have emphasised an assumed morphogenetic simplicity, and ecophenotypic variation. I demonstrate that *C. masoni* does not conform to the current diagnosis of clade Rangeomorpha. Since I consider *Charnia* to nonetheless belong within the Rangeomorpha, recognition of a sympodial central axis and non-identical branching orders expands the known morphological disparity of the rangeomorphs. Consideration of these new data within a Bayesian phylogenetic analysis resolves *Charnia* as a crown-group metazoan lying outside of crown-Bilateria.

## 5.1 Introduction:

Members of the Ediacaran Macrobiota are increasingly interpreted as early representatives of evolutionary grades from which living animal clades are derived (e.g. Budd and Jensen 2017; Hoyal Cuthill and Han 2018; Bobrovskiy *et al.* 2018), but their precise phylogenetic position is often disputed (see Chapter two), and almost every aspect of their biology remains contentious (reviewed in Budd and Jensen 2017). The rangeomorphs are among the oldest members of the Ediacaran Macrobiota, first appearing in rocks of ~571 Ma (Narbonne and Gehling 2003; Pu *et al.* 2016, where preservation allows, in dense palaeocommunities of many hundreds of specimens (Clapham *et al.* 2003; Mitchell *et al.* 2015; Mitchell and Butterfield 2018, Mitchell and Kenchington 2018).

It has been difficult to obtain sufficient diagnostic characters to determine the affinities of these organisms from the study of morphology alone, and analyses of growth and development in Ediacaran taxa have revealed additional characters with which to distinguish between competing phylogenetic hypotheses (e.g. Chapter two; Gold et al., 2015; Evans *et al.* 2017; Hoekzema et al., 2017). Such characters are also of use in discriminating between hypotheses regarding the evolution of large body size (in which rangeomorphs are invoked [e.g. Hoyal Cuthill and Conway Morris 2017]), some of which are reliant on assumed morphogenetic simplicity and plasticity (e.g. Hoyal Cuthill and Conway Morris 2014, 2017).

*Charnia masoni* is perhaps the most well-known rangeomorph, with specimens described from the UK, Russia, Canada and Australia (Laflamme *et al.* 2007; Wilby *et al.* 2015; Dunn *et al.* 2018b). As such it represents an ideal model taxon with which to investigate rangeomorph morphogenesis. In Chapter four, I produced an anatomical model that has been assessed across many populations of *C. masoni*, and so it provides a robust framework within which to base these further analyses. I examine 47 specimens from Charnwood Forest, UK, in order to quantify changes in morphology (associated with changes in size) across development. This is supplemented by the study of four newly collected three-dimensionally preserved specimens of *C. masoni* from the Lyamtsa Formation, Russia, which provide further anatomical and ontogenetic information. These analyses suggest a more complex developmental programme, with temporally and regionally specified growth, than hitherto assumed.

Using this new information, I undertake a phylogenetic analysis to resolve the position of *Charnia masoni*, the results of which indicate that *C. masoni* is a stem-eumetazoan, with statistical tests allowing us to refute non-crown-metazoan and crown-bilaterian alternatives.

## 5.2 Materials and methods:

I examined casts of 47 specimens of *Charnia masoni* from Bed B, North Quarry, Charnwood Forest, UK. Specimens are housed at the British Geological Survey, Nicker Hill, Keyworth. Specimens were not retrodeformed, following Wilby *et al.* 2011. Although I considered *C. masoni* specimens from other global localities when producing the morphological model (Chapter four), those additional specimens are not included in my analysis here in order to reduce the potential influence of environmentally driven morphological variability (Kenchington and Wilby 2017; Hoyal Cuthill and Conway Morris 2017). Six of the most complete specimens (Fig. 5.1) spanning the known size range of *C. masoni* were examined using low angle light. First order branch length was taken as a linear measurement (Fig. 5.1). Second order branches were counted on each first order branch of one row. This analysis was not conducted for all 47 specimens as in many cases the lateral margins of specimens or the complete length were not preserved.

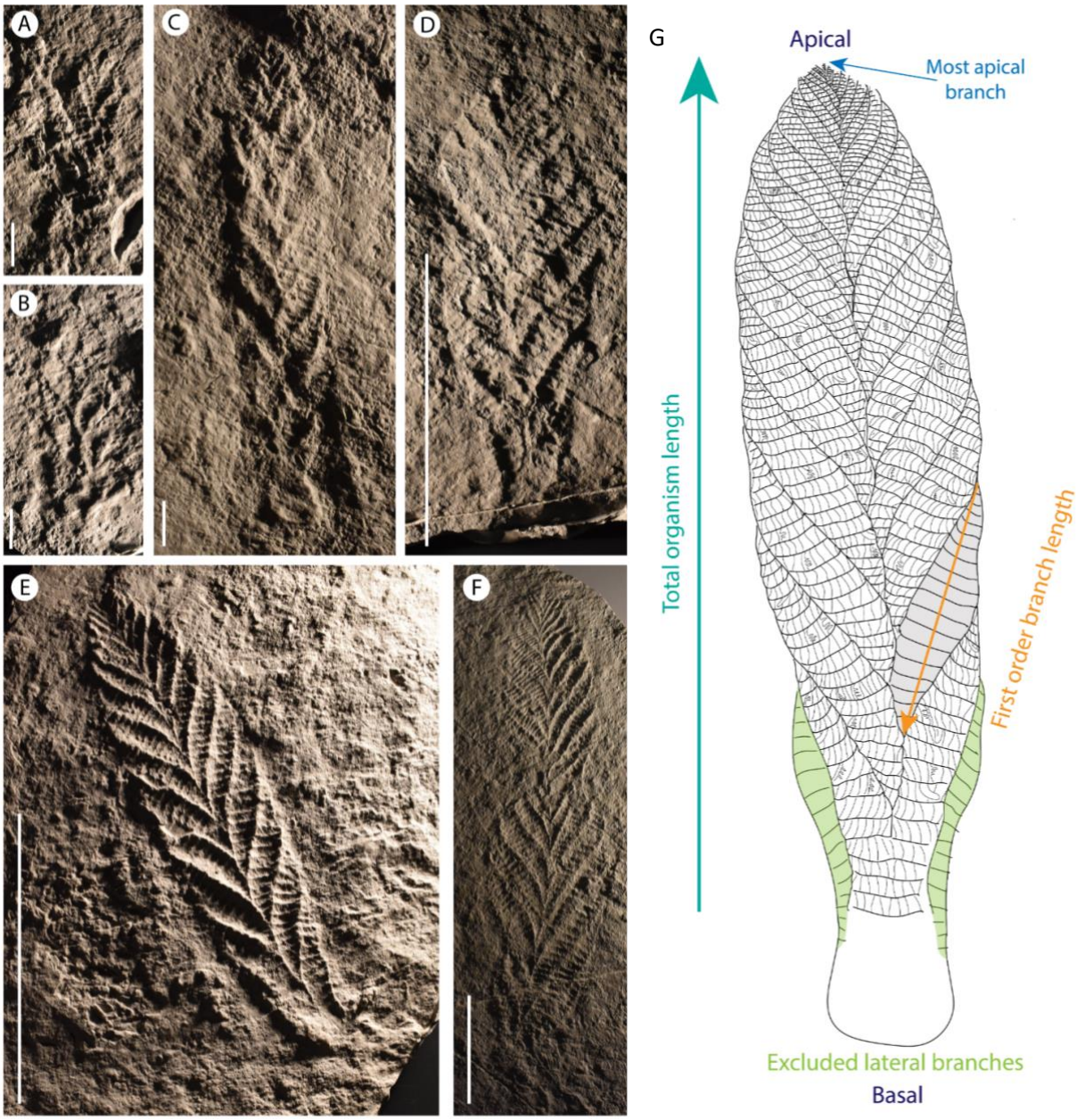


**Figure 5.1:** Six specimens of *Charnia masoni*, ranging from 2.5 – >45 cm used in growth analyses. **A)** GSM 105944, **B)** GSM 106084, **C)** GSM 105989, **D)** GSM 105997, **E)** LEIUG 2328, **F)** GSM 105873. Scale bars in **A–C** = 10 mm, in **D–F** = 10 cm. **G)** Indication of measurements taken when analysing specimens of *Charnia masoni* from Charnwood Forest. Lateral branches (in green) were not included in the analyses.

Growth was interpreted against a null model of self-similar morphogenesis (*sensu* Hoyal Cuthill and Conway Morris 2014).

I assume that:

1) Branches could differentiate during growth.
2) Branches could become larger, but could not deflate or become smaller once formed: some variation in branch architecture (and resultant branch size) has recently been described as ecophenotypic, so only branch orders that showed a stable branch architecture arrangement between and across populations of *C. masoni* (presented in Chapter four) were assessed quantitatively. There is no available evidence to suggest that rangeomorphs were able to actively modulate branch size (e.g. hydrostatically during life). Recent data suggest that some rangeomorphs could alter morphology from concealed to displayed at certain branch orders (Mitchell *et al.* 2018), but it is unclear at present whether this reflects a biological function or a taphonomic artefact.
3) Total organism length varied only due to growth during life. Inflation or deflation of the holdfast theoretically remains a possibility, but there is no evidence to support such a theory. Previous work has shown a predictable relationship between frond length and number of first order branches (e.g. Laflamme *et al.* 2007; Wilby *et al.* 2015) which would not be expected if the frond was able to modulate its size independent of branch growth (e.g. hydrostatically).
4) All members of a single species follow a similar growth plan, although I acknowledge there may be morphometric ecophenotypic variation (as described in Chapter four).

Model choice:

1) My null model is of isometric, self-similar morphogenesis, since previous studies of rangeomorph growth have predicted an isometric, fractal style of morphogenesis (Hoyal Cuthill and Conway Morris 2014).
2) Logistic growth was not tested for: This growth pattern, while commonly observed in population growth (Tsoularis and Wallace 2002), is not typical in single organisms.
3) Exponential growth was not tested for: This growth pattern, while commonly observed in population growth (Tsoularis and Wallace 2002), is not typical in single organisms.

Four three-dimensionally preserved specimens of *Charnia masoni* from the Lyamtsa Formation, White Sea (held at the Central Siberian Geological Museum, specimens CGSM 2079-100-102, 106) were analysed using microfocus X-Ray tomography or synchrotron radiation X-Ray microtomography. Microfocus X-Ray tomography was conducted at the University of Bristol, using a Nikon XTH-225-ST instrument with a Tungsten target with a 0.5mm thick copper filter, a current of between 147 and 156 µA, and voltage of 215kV; the ensuing data were reconstructed using Nikon CT pro 3D. Synchrotron radiation X-Ray microtomography was conducted at the X02DA TOMCAT beamline of the Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland (Stampanoni *et al.* 2007). Specimens were measured using a LuAg:Ce100µm or LuAg:Ce 20µm scintillator and a x4 objective lens (yielding reconstructed tomographic data with 1.625 µm voxel dimensions), at energy levels of 25-30 keV and exposure times of 250-700 ms. 1501 projections were obtained equianglularly through 180$^{o}$ of rotation within the beam. Projections were post-processed and rearranged into flat- and dark-field-corrected sonograms; reconstruction was performed on a 60-core Linux PC farm, implementing an optimized routine based on the Fourier transform method and a regridding procedure (Marone and Stampanoni 2012). Slice data were analysed and manipulated using Avizo 9.4 (FEI). These specimens are not interpreted to have been subject to significant deformation (Stankovsky *et al.* 1990; Grazhdankin 2003, 2004), and are not found in association with independent strain indicators, and so they have not been retrodeformed.

Backscatter electron microscopy (BSE) and energy dispersive X-ray spectroscopy (EDXA) was carried out at the British Geological Survey, Keyworth. Analyses were conducted on an FEI Company Quanta 600 environmental scanning electron microscope with an Oxford Instruments INCA Energy 450 energy-dispersive X-ray microanalysis system using a 50mm$^2$ Peltier-cooled silicon drift ray detector in low vacuum mode. A single second order branch (from specimen CGSM 2017-105) from a three-dimensionally preserved specimen of *Charnia masoni* from the White Sea of Russia was embedded in Epotek 301 resin and polished using polycrystalline diamond paste to 1µm. The accelerating voltage was 15kV, the working distance was 10mm and the diameter of the probe was 563µm.

A complete specimen list can be found in Appendix one.

Morphological phylogenetic analysis was conducted to establish the relationships of *Charnia masoni* among extant opisthokonts. The character matrix was assembled from published matrices (Ax 1994; Nielson 2012; Deline *et al.* 2018), with remaining characters defined from the recent literature (See Appendix two). I followed the multistate coding strategies outlined in Brazeau (2011). Since probabilistic approaches are considered to provide more accurate results than parsimony-based approaches when analysing discrete morphological data (e.g. O'Reilly *et al.* 2016, 2018; Puttick *et al.* 2019), my analyses were implemented in a Bayesian framework, using MrBayes 3.2.6. A gamma distribution was used to model rate variation. Analyses were run for one million generations, sampling every one hundred generations. 25% of trees were removed as burn-in, resulting in 7500 sampled trees. Convergence was assessed by ensuring that an effective sample size larger than 200 was reached, and that the deviation of split frequencies was less than 0.01.

To establish the statistical significance of these results I undertook a steppingstone analysis. Backbone constraint trees resolving *Charnia masoni* in different positions within the tree were produced using the R package 'Paleotree' (Bapst 2012), and then the data-fit to these phylogenetic hypotheses was compared using Bayes Factors. Steppingstone sampling (Xie *et al.* 2010) was used to estimate marginal likelihoods, and the significance of the Bayes Factor difference was established in reference to Kass and Raftery (1995). I ran the steppingstone analyses for 3,100,000 generations, and with 30 steps (producing a stable marginal likelihood), resulting in 1000 samples per step.

## 5.3 Results:

### 5.3.1 Tomographic data

I produced CT and synchrotron scans to investigate whether internal anatomical features were preserved within rare Russian 3D specimens (Figs. 5.2, 5.3). These specimens are preserved as internal molds, with infilling sediment being distinct from overlying sediment but similar to underlying sediment (Fig. 5.3) in terms of porosity and grain sorting. I find that branches are contiguous through the specimen, and show evidence for branch-branch connectivity and so I consider these specimens to faithfully record the anatomy of *Charnia*

*masoni,* as opposed to recording fractures, which may result in completely discrete branch units (Figs. 5.2, 5.3).

In all specimens examined by X-ray microtomography, I find that first order branches are connected to one another, with the basal-most second order branch of a given first order branch directly abutting a second order branch of the ab-basal first order branch (Fig. 5.2D-G), in the absence of a stalk. There is no space between first order branches in which a stalk could be present (Fig. 5.2H-K). First order branches are entirely constructed of second order branches (Fig 5.2A-G). This confers a sympodial central axis on *Charnia masoni* (Fig. 5.2).

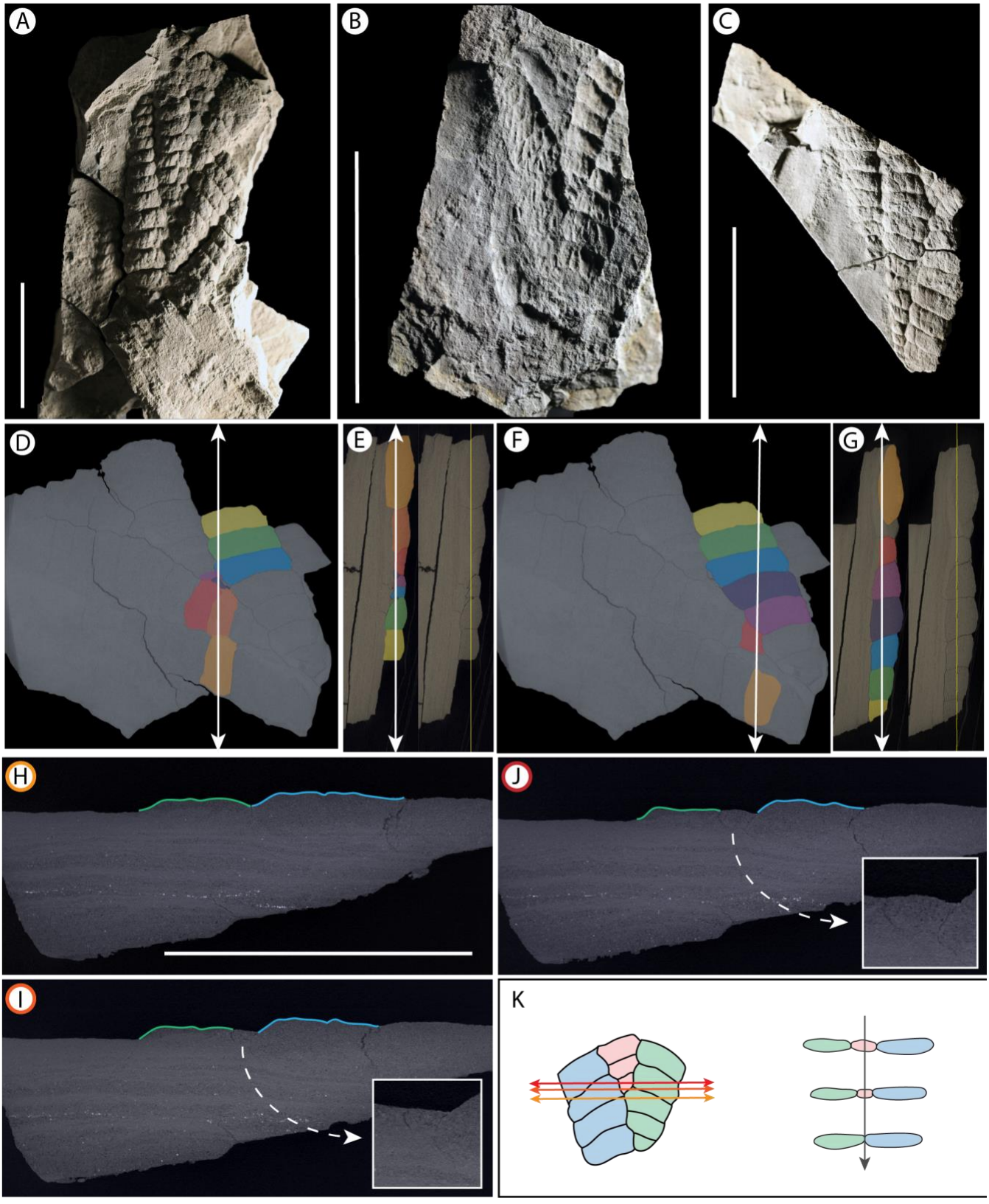
A
B
C
D
E
F
G
H
J
I
K

**Figure 5.2:** *Charnia masoni* specimens from the Lyamtsa Formation, White Sea, Russia, complete specimens and orthoslices after computed tomography. **A-C)** Specimens CGSM 2079-100, CGSM 2079-102, CGSM 2017-101 (Central Siberian Geological Museum). **D-F)** CGSM 2079-100. First order branch interconnections. Coloured branches in **D** and **F** correspond to coloured branches in E and G respectively, with original orthoslice image for comparison. White lines in **D** and **F** (showing X and Y planes) correspond to position of orthoslices in **E** and **G** respectively (showing Y and Z planes). No evidence for an internal stalk is seen. **H-K)** GCSM 2079-101. Orthoslice positions correspond to arrows of equivalent colour in **K**. Green and blue lines correspond to two first order branches, with insets in **I** and **J** illustrating the emergence of a first order branch. Again, no internal stalk is visible. Scale bars **A – C** = 5cm. Scale bars in **A-C** = 5cm, **H** = 1cm.

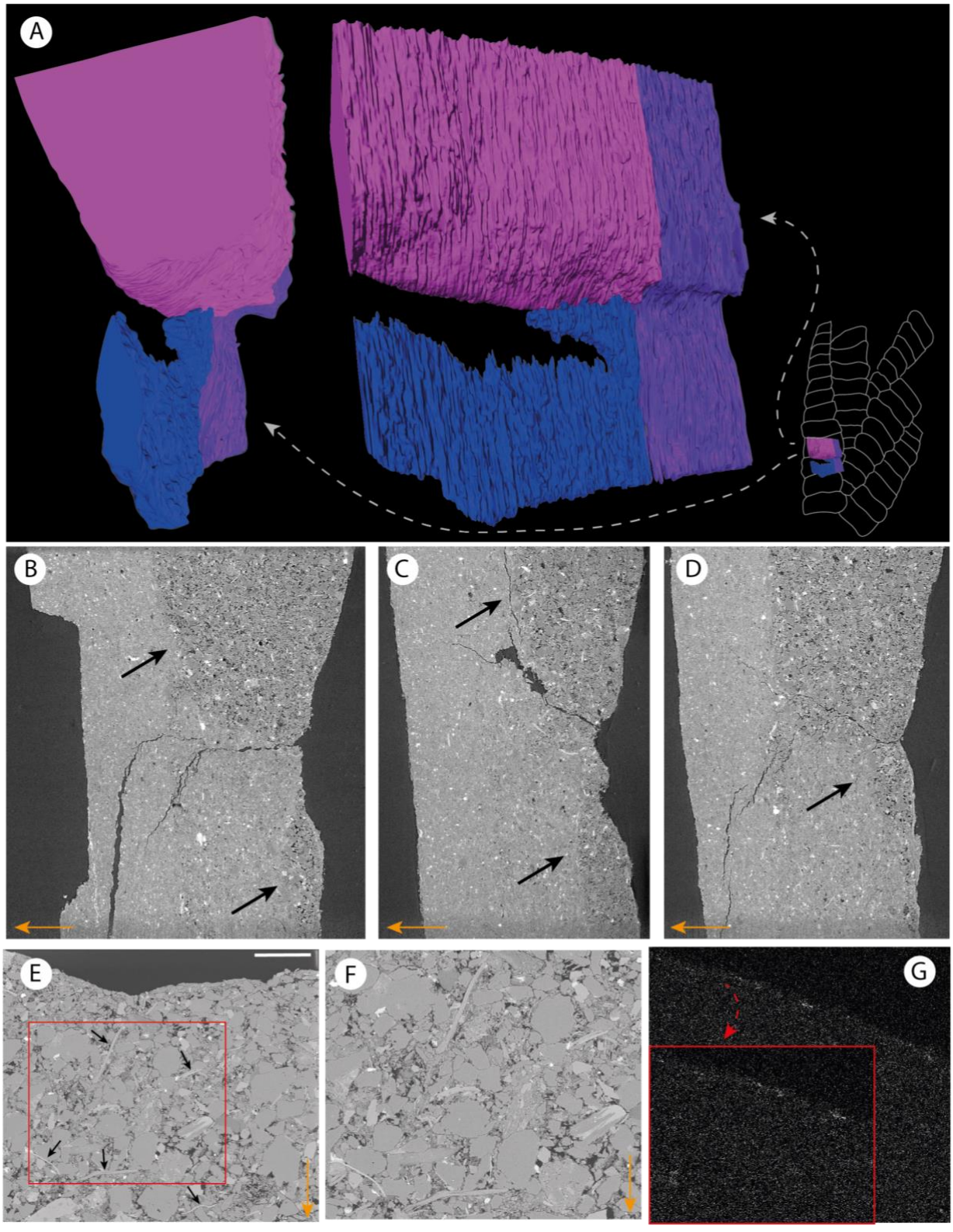
A
B
C
D
E
F
G

**Figure 5.3:** Two second order branches, and the boundary between them. **A)** Rendered model of synchrotron scan of specimen CGSM 2017-105 showing the examined margin between two second order branches. Separate branches are coloured in pink and blue, and an area of apparent connectivity is shown in purple. **B-C)** The individual nature of second order branches shown in individual orthslices. Branches are indicated by black arrows in both cases. **D)** The area of apparent connectivity between second order branches. **E-F)** The 'top' surface of an individual second order branch imaged by back-scatter electron diffraction. Smaller grains are present at the distal margin of second order branches, with individual mica grains at different orientations shown with black arrows in **E**, enlarged in **F.** Image showing different atomic densities; relative high atomic weight in white and low atomic weight in black **G)** The distal margin of a second order branch is slightly enriched in aluminium-bearing minerals, as visualised by EDXA, with greater element abundance shown by brighter colours. Red box shows enlarged area with slight aluminium enrichment. Orange arrows indicate 'top' surface orientation.

In specimens examined using synchrotron radiation X-Ray microtomography there is no evidence for internal structures in any of the second order branches, or grains aligned against the remains of internal structures (Fig. 5.3). Branches are differentiated from surrounding sediment by infill of a higher density (Fig. 5.3B-D); branches consistently display poorly sorted grains, with high porosity, while surrounding sediment is predominantly fine-grained and has low-porosity (Fig. 5.3B-D). When second order branches meet, the boundary is marked by heterogeneity in grain size between the two branches.

The sedimentary branch fill is largely homogenous, except at the 'lower' surface of the branch, which is marked by a shallow layer of fine-grained, well-sorted sediment (Fig. 5.3E-F). This layer is separated from the poorly-sorted fill by a zone of weak enrichment in aluminium-bearing minerals (Fig. 5.3G). This may suggest that the fine sediment was deposited at a different time to the rest of the infilling sediment. This fine-grained layer is not of constant thickness, suggesting that it does not correspond to a biological structure, but rather is the result of incomplete sedimentary infill. Futher, mica grains in the branches do not appear to be aligned with bedding, whereas they are in the surrounding sediment (Fig. 5.3E-F). This suggests that the sediment was not incorporated into the branches slowly, but was incorporated quickly without leaving time for the mica grains to settle. At present, only one specimen has been subject to these investigations, and so a greater sample size is required to confirm or refute these sedimentological findings.

Individual second order branches possess defined internal margins (Chapter four); they do not appear connected to each other across their entire margin (Fig. 5.3A). However, they may be connected to each other medially (Fig. 5.3A). Second order branches overlie each other apico-basally (Fig. 5.3), concurring with the results of Grazhdankin *et al.* 2008.

First order branches abut the ab-basal branch between the third and fifth second order branch across the known size range, where preservation allows quantification (Table 5.1). First order branches typically meet each other in the body of a second order branch, but may sometimes meet at the boundary between two second order branches. Whether this represents *in vivo* biology, or reflects taphonomic alteration is unclear.

### 5.3.2 Growth data

*(a) First order branches*

I quantified the number of first order branches per specimen and compared the relationship between the total length of a specimen and the position of a first order branch along a baso-apical axis and assessed the veracity of two previously published hypotheses on this subject. Hoyal Cuthill and Conway Morris 2014 considered that *Charnia masoni* followed a pattern of self-similar and fractal morphogenesis, while Hoekzema 2016 (unpublished DPhil thesis) suggest that the position of the longest first order branch relative to the poles is at 38% total organism height.

Smaller specimens of *Charnia masoni* possess fewer first order branches than do larger specimens, with the number of first order branches increasing linearly with specimen size across the known size range (Fig. 5.4A). This model predicts that during the known stages of the *C. masoni* life cycle, the organism would not have possessed fewer than 6 first order branches (across both rows of branches). The smallest specimen from Charnwood Forest included in this study (2.6cm total length) bears nine first order branches.

Across most of the body of the frond, first order branches are shorter in smaller specimens than in larger specimens (Fig. 5.5A). This is not the case with the apical-most branches, which remain consistent in length in specimens with total lengths between ~2.6 and 22cm (Fig. 5.4C). In a single specimen of 45cm in length, the most apical branch is five times longer than those of other specimens (Fig. 5.4D). There is no evidence for size-outlying branches that alter the frond outline (*sensu* Kenchington *et al.,* 2018) in any examined specimen from any locality. Furthermore, the position of the longest branch appears to move relative to the poles as the organism increased in total length, from 36% the length of the frond, to 44% and then returning to 38%.

First order branches bear three further branch subdivisions (see Chapter 4). In the largest specimens, higher branch order identification is not limited by the quality of preservation, but no evidence for fifth order branches was observed. However, higher branching orders are often not easily identified near the apex of the frond and so I have not been able to resolve whether four branching orders are present in the most apical branches in all specimens.

Specimens up to ~5cm total length show a linear relationship between first order branch number and branch length (Fig. 5.5B, C). All larger specimens show a non-linear relationship between branch number and branch length (Fig. 5.5D-G). In specimens between 11 and 15cm, this relationship was best explained by a second order polynomial regression; in the holotype specimen (22cm) this relationship was best explained by a third order polynomial regression; in the largest examined specimen (45cm in length) this relationship was best explained by a second order polynomial regression. In all cases model fit was 0.85 or higher.

*(b) Second order branches:*

The number of second order branches per first order branch was quantified and compared to the relationship between the length of a first order branch and its position along a baso-apical axis (Fig. 5.4B). The number of second order branches in the apical-most first order branches was rarely quantifiable due to preservational constraints (i.e. features were too small to be preserved/observed). In specimens where it was possible to quantify either the most apical branch or penultimate apical branch, the number of second order branches is lower than across the rest of the frond (Fig. 5.5H).

The number of second order branches in a given first order branch is broadly greater in larger specimens than in smaller ones (Fig. 5.5H). There is no clear relationship between first order branch size within a specimen and the number of second order branches they bear (most apical branches excluded).

Estimates for the maximum number of second order branches a first order branch may host increase linearly with specimen size (Fig. 5.4B). This model predicts that throughout the frondose stage of the *Charnia masoni* life cycle, each first order branch did not host fewer than eight second order branches. This does not include the most apical branches. The maximal number of first order branches per frond, and maximal number of second order branches per first order branch, are both explained by a linear regression, but exhibit different slopes (0.74 and 0.32 respectively).

| Specimen accession code | branch pair number | Number of second order branches beneath first order branch emergence |
|---|---|---|
| CGSM 2079 -102 | ? | 3-4 |
| CGSM 2079 - 100 | ? | 3 |
| | ? | 4-5 |
| GSM 106160 | 1 | 4-5 |
| | 2 | 5 |
| | 3 | 4 |
| | 4 | 5 |
| | 5 | 5 |
| | 6 | 5 |
| | 7 | - |
| | 8 | 5-6 |
| | 9 | 5 |
| | 10 | 4 |
| | 11 | 4 |
| GSM 105873 | 2 | 3-4 |
| | 3 | 4 |
| | 4 | 4-5 |
| GSM 105989 | 3 | 4 |
| GSM 105877 | 2 | 3 |
| | 3 | 3 |
| | 4 | - |
| | 5 | 3 |
| GSM 105977 | 4 | 4 |
| | 5 | - |
| | 6 | 5 |
| GSM 105873 | 4 | 3-4 |
| | 5 | 5 |
| | 6 | 6 |
| | 7 | 4 |
| | 8 | 5 |
| | 11 | 4 |
| | 12 | 3-4 |
| | 16 | 4-5 |
| | 17 | 3 |
| | 18 | 4 |
| GSM 106084 | 3 | 4 |

**Table 5.1:** The number of second order branches per first order branch that lie basally/proximally to the meeting point with a second first order branch. Where two numbers are indicated, the two first order branches meet at the second order branch boundary, and so may be associated with either branch. Where a sequence of branch boundaries is preserved well enough to be reliably quantified, the sequence is presented with a dash indicating missing data.

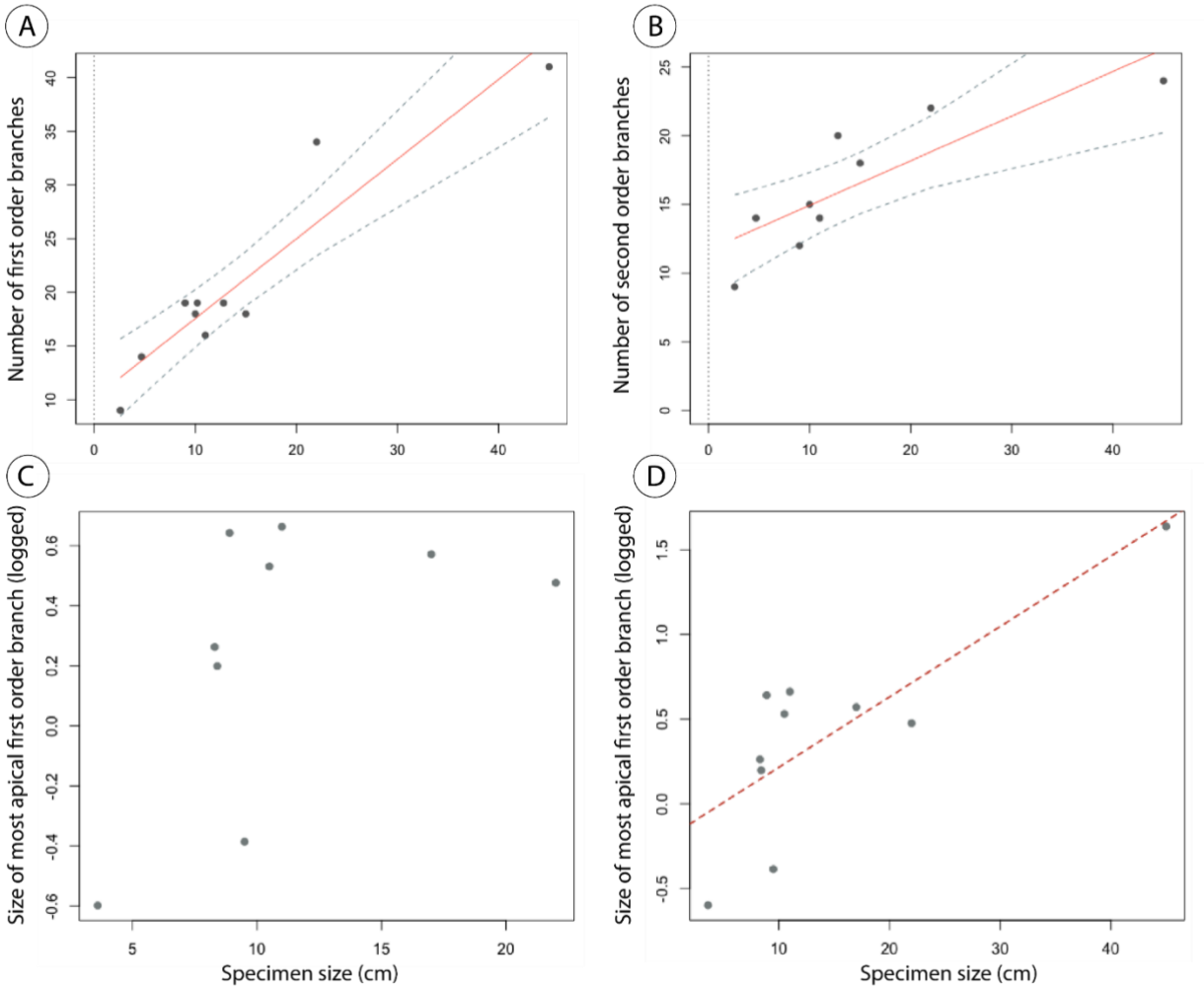


**Figure 5.4: A-B)** The relationship between specimen length and the number of first and second order branches present amongst the *Charnia masoni* population from Charnwood Forest. **A)** Linear relationship between the total number of first order branches and size of specimen, P = $5.45*10^{-5}$ (orange unbroken line). Intercept for 2.5% confidence bar = 6.11 and intercept for 97.5% confidence bar = 14.2, grey dotted lines. **B)** Linear relationship between the maximal number of second order branches (minimum estimate) per first order branch, and the size of a specimen, P = 0.004 (orange unbroken line). Intercept for 2.5% confidence bar = 8.14 and intercept for 97.5% confidence bar = 15.2, grey dotted lines. **C-D)** The relationship between the total length of the most apical branch and the total specimen size (length of most apical branch logged). **C)** Omitting the largest specimen: no significant relationship between the variables. **D)** Significant relationship (P =0.003, orange dotted line) for a linear model when the outlying specimen is included.

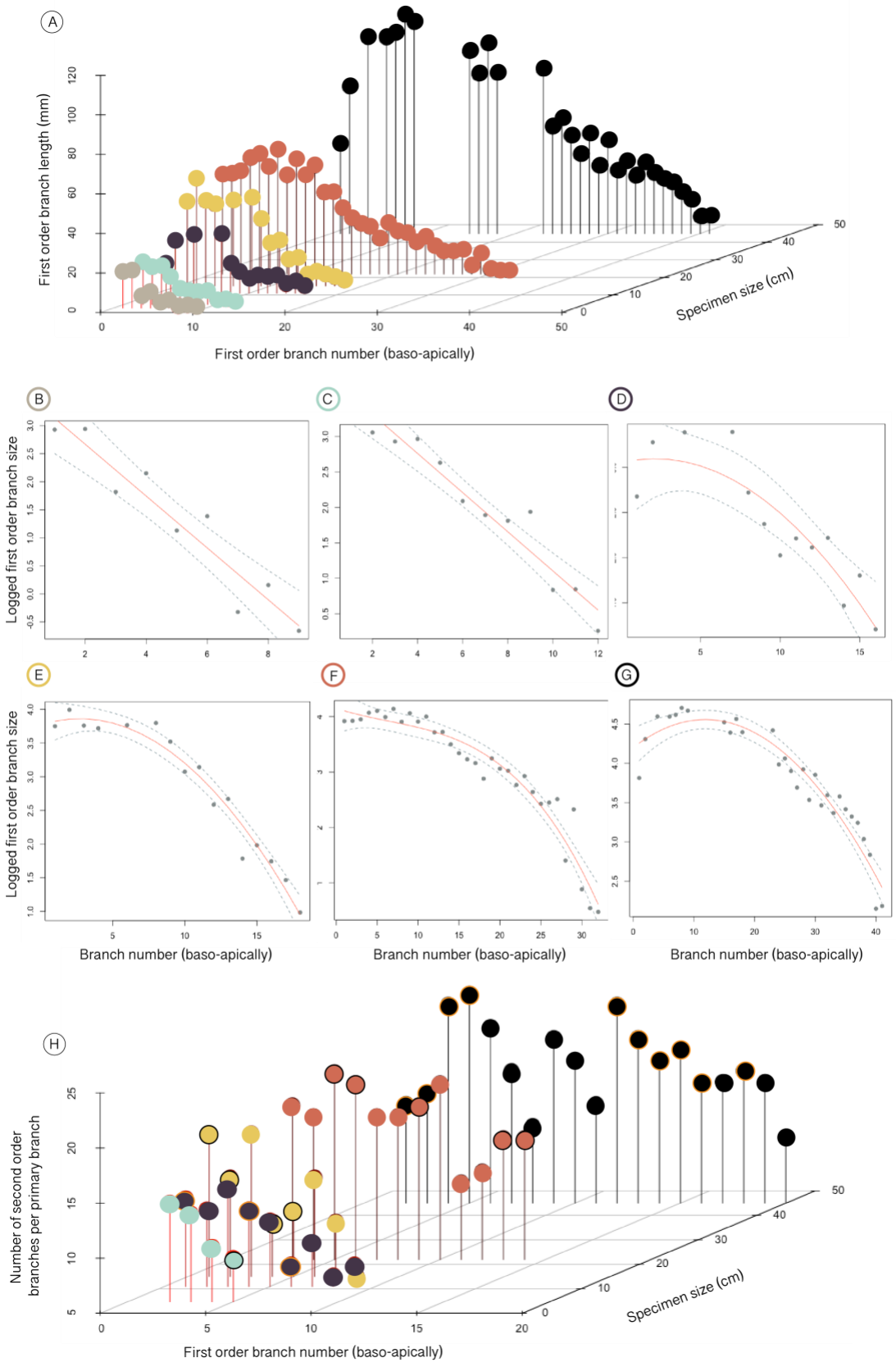
A
First order branch length (mm)
First order branch number (baso-apically)
Specimen size (cm)
B
C
D
Logged first order branch size
E
F
G
Branch number (baso-apically)
H
Number of second order branches per primary branch
First order branch number (baso-apically)
Specimen size (cm)

**Figure 5.5:** The relationship between the number and position of first and second order branches along a *Charnia masoni* specimen, and its total length. **A)** 3D plot showing six specimens of *C. masoni* spanning the known size range. **B-G** best fitting model with 95% confidence intervals for all specimens plotted in **A,** with the colour of the panel label outline indicating the respective specimen from **A**. All data have been logged. **B)** Specimen size = 2.6cm. Best supported model = Linear model, P = $7.35e^{-05}$, adjusted R squared = 0.89. **C)** Specimen size = 4.7cm. Best supported model = linear model, P = $1.89^{e-06}$, adjusted R squared = 0.92. Longest first order branches = 36% up length of frond (baso-apically). **D)** Specimen size = 11cm. Best supported model = second order polynomial model, P = 0.0003, adjusted R squared = 0.85. Longest first order branches = 44% up length of frond. **E)** Specimen size = 15.1cm. Best supported model = second order polynomial model, P = $3.58e^{-10}$, adjusted R squared = 0.96. Longest first order branches = 44% up length of frond. **F)** Specimen size = 22cm. Best supported model = second order polynomial model, P = $<2.2e^{-16}$, adjusted R squared = 0.93. Longest first order branches = 38% up length of frond. **G)** Specimen size = 45cm. Best supported model = second order polynomial model, P = $2.94e^{-10}$, adjusted R squared = 0.91. Longest first order branches = 41% up length of frond. Missing data (branches insufficiently preserved to measure accurately) were omitted. **H)** The relationship between number of second order branches per first order branch and specimen size. Circles with outlines represent a minimum estimate of number of second order branches per first order branches.

## 5.4 Discussion:

*Charnia masoni* has four orders of branches. These branching orders may be rotated and furled (as with the first two branching orders), or appear rotated or displayed, furled or unfurled, undivided (as with the third branching order), or current-swept (*sensu* Brasier *et al.* 2012), as discussed in Chapter four. First order branches have previously been hypothesised to be arranged around a central stalk (Narbonne 2004; Laflamme and Narbonne 2008a; Narbonne *et al.* 2009; Hoyal Cuthill and Conway Morris 2014), but data presented in this study demonstrates this is not the case, and, rather, first order branches emerge directly from each other.

### 5.4.1 A model for the growth of *Charnia masoni*:

Following Chapter four, the lateral branches are the most basal pair of first order branches across the frond, connecting directly to the holdfast of the organism, rather than to the other first order branches. Therefore, these branches are not associated with the same sympodial central axis as the rest of the first order branches, and I cannot infer their relative growth compared to the rest of the frond. Because the lateral branches are distinct in this way I may exclude them from my growth studies without biasing my opinion of how the main frond developed. However, I consider them to represent a disctinct growth zone (as discussed in Chapter 4). I conclude that the visible branch architecture for first and second order branches is representative of original anatomy, rather than taphonomic artefact. This is because the anatomy from two-dimensional specimens is matched by the anatomy of three-dimensional specimens presented here, and neither show evidence for any rotational variation. This is unlike the rotational variation of third and fourth order branches, which differentiate from second order branches.

First order branches are serially stacked, with the central axis of the organism derived from these successive lateral branches, conferring a sympodial central axis on the organism. Differentiating first order branches result from outgrowth from the basal region of an existing first order branch. There appears to be consistency in the number of second order branches below the emergence of the adapical first order branch between specimens, perhaps indicating that the area of first order branch differentiation is pre-determined.

No evidence for any stalk or stalk-like structure was found associated with second order branches (see Fig. 5.3A), and they appear to connect directly to each other medially (second order branch axis; Dunn *et al.* 2018b; Fig. 5.3A). The arrangement of second order branches stacked on top of each other is suggestive of budding outgrowth (from a more basal second order branch), but further evidence is required to confirm this hypothesis.

The presence of sediment filling the interior of *Charnia masoni* branches necessitates at least one opening in the exterior of the organism through which sediment can enter. Sediment may have either infilled the organism during life, or post-mortem. No other rangeomorph frond from any locality are described as filled with sediment during life, although note that the holdfast – termed axial bulb – of *Rangea schneiderhoehni* is interpreted as sediment filled in life; Vickers-Rich *et al.* 2013). However, I note the capacity for certain sponges to incorporate sediment (e.g. Cerrano *et al.* 2004) during life, although this is typically for skeletal support and while some sponges do possess sediment pockets, sediment does not fill the entire body (Schöenberg, 2016). The sedimentary data presented above suggests the frond of *C. masoni* was filled with sediment rapidly and completely, unlike what is known in living sponges. I therefore consider it unlikely the frond of *C. masoni* was filled with sediment during life.

A sympodially organised central axis requires that adapical branches are younger than abapical branches because they derive from them. This is corroborated by evidence showing that the apical-most first order branches are size invariant across much of the variation in frond size, whereas more basal branches vary in accordance with frond size (Figs. 5.5, 5.6). The relationship between the maximal number of first order branches and the size of the specimen suggests either a cryptic non-linear phase of branch differentiation, or that *Charnia masoni* branches differentiated from a non-frondose precursor.

Given this, I consider the outline of each frond (dictated by the length of first order branches) to represent a growth series, with basal branches necessarily being older than apical branches. Initially, the length of a first order branch is directly proportional to its position along the apico-basal axis, resulting in a linear frond outline. In larger fronds, a non-linear outline indicates that first order branch size is not (seemingly) only correlated to position along an apico-basal axis.

First order branches are entirely constructed of second order branches. The number of second order branches per first order branch is lowest in the most apical branches, but remains stable across the rest of the body of the frond. This confirms that first order branch size changes are not exclusively driven by the differentiation of second order branches, but by the 'inflation' of pre-formed branches (suggested as a possibility by Flude and Narbonne 2008).

There are fewer second-order branches in the most apical first order branches across all examined specimens, suggesting that first order branches do not differentiate with a complete set of second order branches. There must then be a period of non-linear second order branch differentiation during which this early differentiation occurs. Given there is consistency in the number of second order branches basal to a first order branch along the sympodial axis, it seems unlikely that new second order branches differentiate basally. It remains possible for second order branches to differentiate from a generative zone just apical to the first order branch generative zone, but in the absence of any evidence for this (i.e. unusually small second order branches in this region) I view this as unlikely. Therefore, I consider it most likely that second order branches differentiate from the apex of the first order branch, with the respecification of the generative zone in emerging second order branches.

The most apical branches in the largest specimen are not size equivalent to the most apical branches in smaller specimens, but are larger than the correlation between specimen size and apical branch length predicts (Fig. 5.4C-D). This may suggest that a maximum number of first order branches was reached/being reached (*sensu* Wilby *et al.* 2015). Thus, while growth in smaller specimens was dominated by differentiation, in larger specimens differentiation of branches either ceased or slowed. I discriminate stages in the *Charnia masoni* life cycle between a first stage in which the branch differentiation takes place - termed the 'differentiating frond' - where frond growth occurs via both the differentiation of new units and their subsequent inflation, and a second stage termed the 'inflating frond', whereby differentiation ceased or slowed. These stages appear to represent different developmental modes. The largest specimens of *C. masoni* are incomplete and so their full dimensions remain unknown. Nevertheless, the available data are compatible with an indeterminate growth strategy (Wilby *et al.* 2015; Hoyal Cuthill and Conway Morris 2017).

Morphological variability of third and fourth order branches compromises quantitative analysis of their growth, but morphological data allow for some inference. There is no evidence to suggest a continuation of third order branch margins into the interior of second order branches. In Chapter four, I demonstrated that third and fourth order branches may show the full range of rangeomorph branched architectures (undivided – displayed), they may appear 'swept' at their apical margin, and they may overlay each other.

Together, these data suggest that *Charnia masoni* possessed a level of temporally-controlled and regionally-specific change, indicating a level of developmental pre-determination hitherto assumed absent. Indeed, the pattern of growth my data imply shows variability between first order branches. Therefore, my results contradict those of Hoyal Cuthill and Conway Morris 2014 who model the growth of rangeomorphs as following a self-similar and fractal style of morphogenesis (Hoyal Cuthill and Conway Morris 2014; Hoyal Cuthill and Conway Morris 2017). Furthermore, my results contradict those of Hoekzema 2016 (unpublished DPhil thesis), who predicts a stable position for the longest first order branch at 38% the length of the frond. This model suggests that *C. masoni* conforms to the 'golden ratio', (0.38 versus 0.62), common in the growth programmes of many organisms including plants.

My data undermines hypotheses concerning the origination and diversification of rangeomorphs that are reliant on a plastic morphology (in discrete and continuous characters). This model is summarised in Fig. 5.6.

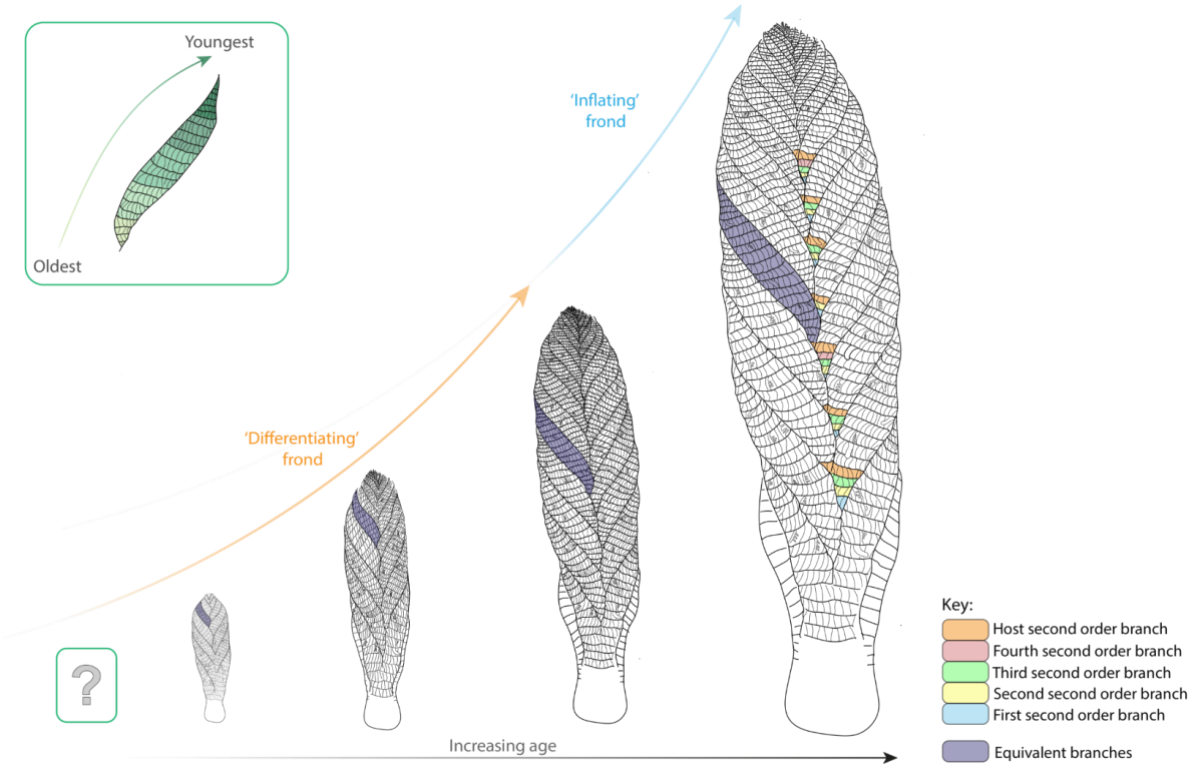


**Figure 5.6:** A model for the morphogenesis of *Charnia masoni.* Green box representing the unmodelled stage in the *C. masoni* life cycle. The differentiating and inflating frond are illustrated, along with changes in branch measurements such that the basal-most branches are longest in the smallest illustrated specimen, and this transitions towards the middle of the frond with increasing size. The consistent relationship between second order branch number (in a given first order branch) is illustrated in the largest schematised *C. masoni* specimen. A single first order branch (equivalent branch in key) is traced through all growth permutations illustrated, and the presumed growth trajectory of second order branches is shown in inset. What preceeds the smallest known frondose stage is unknown, and marked by a question mark.

### 5.4.2 Comparison to other rangeomorphs

*Charnia masoni* has, historically (e.g. Antcliffe and Brasier 2007) and in this thesis, been used almost as an archetypal rangeomorph against which others are considered, under the assumption that trends described in *C. masoni* are reconcilable with those in other rangeomorphs. Variation in branching type and morphogenesis has been documented in a number of rangeomorphs (summarised in Chapter two) and this must be considered alongside data from *C. masoni* if we accept rangeomorphs as a natural grouping (e.g. Dececchi *et al.* 2017, 2018; Hoyal Cuthill and Han 2018).

First order branches are entirely constructed of second order branches, and second order branches host third and fourth order branches (but, crucially, do not appear to be entirely constructed of them as they are able to move independent of their second order branch and so make up only a component of it). I interpret the second order branch as the fundamental repeated unit of *Charnia masoni* because it is the constructional unit upon which all other branching orders are either constructed of (first order branches) or are derived from (third and fourth order branches). The differences in first and second order branch anatomy as compared to third (and fourth) order branch anatomy means that *C. masoni* does not conform to the definition of Rangeomorpha (Erwin *et al.* 2011, SOM), which requires three orders of self-similar branching. This definition, while erected on best knowledge at the time, may have had unintended consequences in limiting the possible anatomical permutations considered possible for this group.

Subsidiary branches lying between 'frondlets' in the rangeomorph genus *Fractofusus* (Gehling and Narbonne 2007) imply that multiple, secondary growth zones can be present in rangeomorphs, as may be the case with the generation of the lateral branches in *Charnia masoni*. *Hylaecullulus fordi, Primocandelabrum* and *Bradgatia* exhibit 'eccentric branching', an overcompensatory branch response (Kenchington *et al.,* 2018). These multifoliate taxa with multiple rows of first order branches have a bush-like appearance. Eccentric branching has been suggested to confirm a modular construction of rangeomorph taxa, consistent with the serially organised branching orders of *C. masoni.* The eccentric branching response is never observed beyond the second branching order. This may suggest that the highest branching orders in three other rangeomorph genera (*Hylaecullulus, Primocandelabrum* and

*Bradgatia*) are unable to act independently of their host second order branch. If the first order branches are constructed entirely of second order branches (no internal stalk has been reported nested within the branches of these taxa) then this may provide further evidence that the second order branching unit is the fundamental repeated unit in rangeomorphs.

Variation in rangeomorph growth strategies does not appear fundamentally incompatible with the interpretation that rangeomorphs comprise a clade (Dececchi *et al.* 2017, 2018). However, there is insufficient anatomical and developmental information available for many rangeomorphs, and continued taxonomic uncertainty surrounding another frondose Ediacaran group (Arboreomorpha) means that currently it is not possible to erect a new definition encompassing new morphological characters for the Rangeomorpha that satisfactoraily differentiates them from the arboreomorph genus *Charniodiscus*. If the second order branch does reflect the fundamental repeated unit in the rangeomorphs, an emended definition should look to characterise the anatomy of this element.

These findings have bearing on how we consider homology between different rangeomorph taxa; whether *Charnia masoni* is homologous with an entire *Hylaecullulus fordii* organism (with one first order branch being homologous to a folium, or whether *C. masoni* is homologous to a single folium of *H. fordii.* Antcliffe and Brasier (2009), consider entire organisms as homologous because those authors view transitions between undivided and completely displayed branching architectures as taxonomically and evolutionarily significant. However, I suggest this may not be the case, with variation in at least higher order branches being the result of rotational variation (Chapter four), and so, below, I readdress the problem of homology.

The first order branches in *Charnia masoni* emerge directly from each other, and so they are intradependent. These branches represent serial homologues (e.g. Wagner 1989), and I must therefore consider the main frond of *C. masoni* as one entity when comparing to other fronds. There are other rangeomorph taxa that do not possess a central stalk in any known specimen (e.g. the genus *Beothukis* Brasier and Antcliffe 2009). Until a central stalk can be demonstrated it is reasonable to consider these forms as sympodially branched, like *C. masoni*. In this case the main fronds of *C. masoni* and *Beothukis* would be homologous. Taxa including *Avalofractus abaculus* and *Pectinifrons abyssalis* (Bamforth *et al.,* 2008; Narbonne *et al.* 2009) have central stalks/rods that host independent first order branches. These

branches necessarily have different originations, and so do not represent serial homologues, and so only one first order branch is homologous to an entire *C. masoni.* Multiple frondose branches can arise from a single holdfast structure (consider the main frond and the lateral branches of *C. masoni*), and so in the absence of evidence to suggest a single origination of folia in multifoliate forms like *H. fordii* or *Bradgatia* (and thus the serial homology of folia), even where a holdfast is present (Boynton and Ford 1995; Kenchington *et al.,* 2018; though see Brasier and Antcliffe 2009 for an alternative interpretation), I consider a *C. masoni* frond to be homologous with a single folium. This scheme is summarised in Fig. 5.7.

This new homology scheme may suggest that rangeomorph interrelationships need to be reconsidered. The benthic reclining *Pectinifrons abyssalis* shows a body plan homologous to that of the uniterminal frond *Avalofractus abaculus*, but distinct from other such forms including *Fractofusus*, where a central stalk is yet to be demonstrated. Until a stalk can be demonstrated, the anatomy of *Fractofusus* can be more readily homologised to forms like *Charnia masoni* and *Beothukis* than to *P. abyssalis* or *Bradgatia*, to which it is generally considered closely related (e.g. Dececchi *et al.* 2017, 2018). This may imply multiple excursions into similar ecospace by different rangeomorph groups, and questions the phylogenetic utility of characters associated with such excursions (e.g. the simple presence or absence of a holdfast disc). Indeed, large inter-taxon variability in holdfast structure may support such a hypothesis (e.g. the bulbous holdfast of *C. masoni* and the flat, plate-like holdfast of *Hylaecullulus,* schematised in Fig. 5.7A).

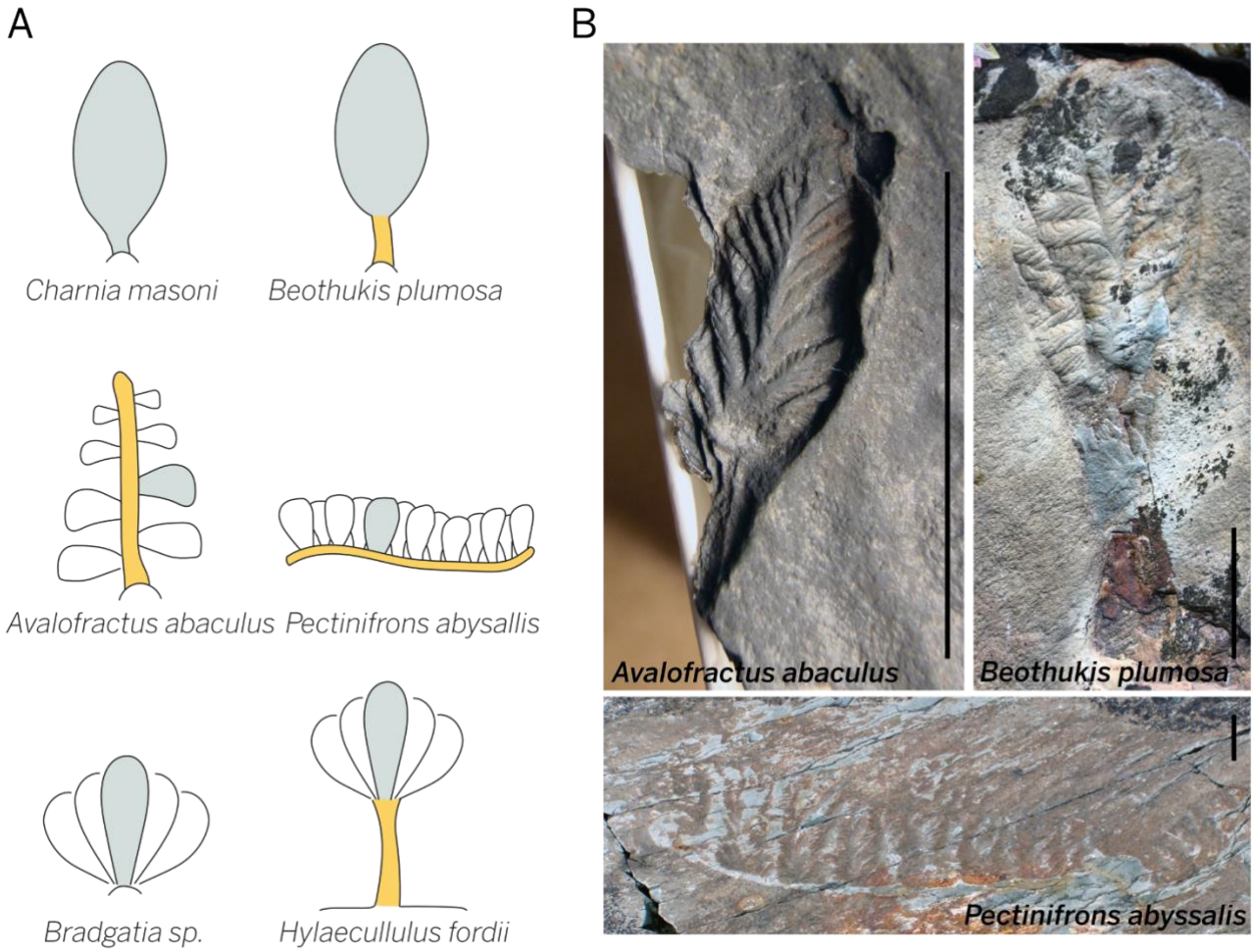


**Figure 5.7: A)** Suggested homology scheme for rangeomorph taxa, where grey represents homologous units. Orange indicates where stalk or stem-like structures are present, though these need not necessarily be homologous. **B)** Examples of fossil taxa from which this scheme is derived. Scale bars in **B** = *Avalofractus* = 2.5cm, *Beothukis* and *Pectinifrons* = 5cm.

### 5.4.3 Branching morphogenesis

Branching organisation is a common body plan across disparate extant and extinct organisms, and several trends in the progression of branching morphogenesis have been documented. Here I summarise where branching morphogenesis is encountered in organs and organisms.

While there are many differences in the intricacies of branch specification across disparate organisms and organs, some have questioned whether there are fundamental similarities in the branching growth observed in, say a lung, a tree and a coral (e.g. Harrison 2010). Where branching morphogenesis has been modelled *in silico,* the simplest forms of ductal branching (in the absence of competition between growing tips) demonstrate symmetrical and stereotypic branching (Hannezo *et al.* 2017). This pattern is not typical in biological branching systems, where a variety of processes and mechanisms mean that there are no paradigmatic real-life examples (Varner and Nelson 2014), although there do appear to be some mechanistic similarities.

Branch specification in bilaterian organ systems largely seems to occur as a result of localised outgrowth, be that differential growth, invasive branching, or epithelial folding (reviewed in Affolter *et al.,* 2009; Varner and Nelson 2014), rather than apoptosis (except in some cases where exact specification of the branch is crucial, e.g. the digits of the vertebrate limb bud [reviewed in Zeller, Zuniga and Lopez-Rios 2009]). Branch polarisation occurs initially at the cellular level, but subsequently by means of the specification of a 'stalk' and a 'tip' in budding growth (e.g. Wang *et al.* 2017). Cells of the tip may act in a leader capacity (as a distal organiser), with stalk cells following their migration. These two domains are kept separated by either lateral inhibition, or cell affinity mechanisms, and early branch extension progresses by tip extension (Affolter *et al.,* 2009). It seems that second order branch growth occurs by tip extension in *Charnia masoni.*

Branching growth in plants, algae and non-bilaterian metazoans appear to show an environmental responsiveness, with the branching form correlated to light availability (Mercado-Molina, Ruiz-Diaz and Sabat 2016; King 1994). In coral species first order branches are known to ramify in spatially congruent positions in different environments (e.g. Sánchez *et al.* 2004, 2007), but the rate of second order branch ramification varies in response to light availability (Mercado-Molina *et al.* 2016). Leaf shape does not appear consistent across

organisms, with local morphogen gradients acting independently (e.g Runions, Tsiantis and Prusinkiewicz 2017). While I can say nothing about rate in the branching morphogenesis of *Charnia masoni*, the morphology of *C. masoni* is consistent (lack of aberrant branches, four orders of branching arranged in the same way) across global sites, suggesting a lack of morphological responsiveness (in discrete characters, e.g. branching state) to ambient changing environmental factors (e.g. nutrient availability) in examined parameters. *C. masoni* appears to have different dimensions in different environments, explored in Chapter four, indicating that the organism was perhaps able to alter branch length and width or total specimen length and width in response to changing environmental factors.

In response to stress or damage, plants, algae and certain corals appear to respond in different ways, with gorgonian corals and some algae producing an overcompensatory branch response, plants specifying multiple new generative zones, and some arborescent bryozoans showing either no response or repairing the original structures. Some rangeomorphs appear to exhibit an overcomensatory branch response (summarised in Kenchington *et al.,* 2018)

Bilaterian branching systems are typically at the organ level (e.g. the mammalian lung, the *Drosophila* tracheal system or the branching of the renal system) and, like entire branching organisms, their morphology has been considered to be generated as a response to 'external' cues (here, morphogens) (e.g. Affolter *et al.* 2009). Growing tips are most commonly arrested by the presence of neighbouring branches, creating density-dependent feedback control (Hannezo *et al.* 2017). I find no evidence for similar controls in *Charnia masoni*, but this remains feasible as a mechanism with which to regulate size dynamics in stacked second order branches.

Charniid branch architecture (the branching architecture which defines *Charnia masoni*, and was previously considered to show undividied branches [*sensu* Brasier *et al.* 2012] at all orders) follows that described by the idealised model of branching morphogenesis (stereotypical and symmetrical branching) in all known specimens, and rangeomorph specimens that do exhibit growth abnormalities are compellingly attributed to damage response (Kenchington *et al.,* 2018). Together, this strongly suggests that rangeomorph branching morphogenesis and morphology (though perhaps not morphometrics; Chapter four) was governed by internal regulation, and was not responsive to ambient environmental conditions (*contra* Hoyal Cuthill and Conway Morris 2017), whereby the branching

architecture is invarient. Charniid branching therefore appears distinct from all known extant comparators.

### 5.4.4 Phylogenetic analyses and inference:

Recently, there have been attempts to integrate *Charnia masoni* and/or other rangeomorphs into phylogenetic analyses to resolve either the inter-relationships of Ediacaran taxa (Dececchi *et al.* 2017, 2018), or the relationship of Ediacaran taxa to extant organisms (Hoyal Cuthill and Han 2018). Dececchi *et al.* (2017) note that there has been little success in attempting to rationalise between Ediacaran taxa and extant groups because characters of assumed phylogenetic significance (i.e. homologous characters) may be convergent in origin. In attempting to overcome this problem, their studies made no assumptions about trait history, effectively removing inference of homology and thus rendering their analyses phenetic rather than phylogenetic. This limits the usefulness of their work for attempting to infer the interrelationships of examined taxa, which would require a phylogenetic approach. Alternatively, Hoyal Cuthill and Han (2018) include characters within their character matrix that are demonstrably non-homologous (e.g. their character 15 - primary striation of zero order units - draws homology between vertebrate sarcomeres, cnidarian tentacles, cnidarian septae, ctenophore ctenes and annelid parapodia), or which are known to be unreliable indicators of organismal relationships (e.g. their character 27 – organismal width/length). These characters undermine the results of the analysis and the inferences the authors attempt to derive from them. Both studies also inappropriately code serially homologous characters (e.g. successive rangeomorph branching orders) as independent (Billet and Bardin 2018).

However, understanding the position of *Charnia masoni* in metazoan phylogeny is important if we are to dienstangle the role it and other rangeomorphs may play in interpreting early animal evolution. I have attempted to overcome the problems set out above by compiling a list of characters from *C. masoni* and representatives of the Holozoa, with the fungus *Laccaria bicolor* used as the outgroup. This phylogenetic bracket was chosen because the developmental characters presented in this chapter for *C. masoni* are a combination of developmental characters only known today in the Metazoa (reviewed in Chapter two), namely the maintained differentiation of elements across development along with

concurrent axially delineated inflation; determinate form across all known life stages; and the presence of ontogenetic shifts.

The ingroup taxon set used includes two non-metazoan holozoans, two poriferans, one placozoan, one ctenophore, three cnidarians, five protostomes and five deuterostomes. *Charnia masoni* was the only fossil taxon included in this analysis. Excepting one, all chosen morphological characters describe extant morphological variation considered to be phylogenetically significant (Ax 1994; Nielson 2012; Deline *et al.* 2018, additional character references in Appendix two). 109 morphological characters were used. One morphological character ("body constructed of repeated multiply-branching units") defines *C. masoni* to the exclusion of the extant comparators. No further aspects of *C. masoni's* anatomy were considered phylogenetically robust for these analyses. All characters requiring cellular or subcellular resolution (e.g. cnidocytes) were recorded as missing in *C. masoni.* Characters that are not present in known specimens but where absence could be an artefact of preservation (e.g. an aquiferous system) were recorded as missing in *C. masoni. C. masoni* was only scored for characters that are demonstrably present or absent, (e.g. *C. masoni* has a body constructed of repeated multiply branching units; *C. masoni* does not have bilateral symmetry). This resulted in *C. masoni* being coded present, absent or not-appicable for 49% of characters. The full character list and data matrix can be found in Appendix one.

My analyses recover *Charnia masoni* as a stem-eumetazoan with 100% posterior probability (Fig. 5.8). I assessed the relative power of the model in discriminating between different phylogenetic hypotheses by comparing Bayes Factors (Table 5.10). Bayes Factors examine the posterior odds of two hypotheses when the prior probabilities are equal. A Bayes Factor of > 10 suggests strong support for a given phylogenetic hypothesis in the criteria set out by Kass and Raftery (1995). I find that a crown-metazoan affinity is strongly supported, and placement within total-group Eumetazoa is substantially supported (Table 5.10). It is possible to reject with strong support a total-group protostome or total-group deuterostome affinity (i.e. crown-Bilateria), but it is not possible to distinguish between the remaining competing phylogenetic interpretations (stem-Eumetazoa, stem- Coelenterata, stem-Bilateria) (Table 5.10).

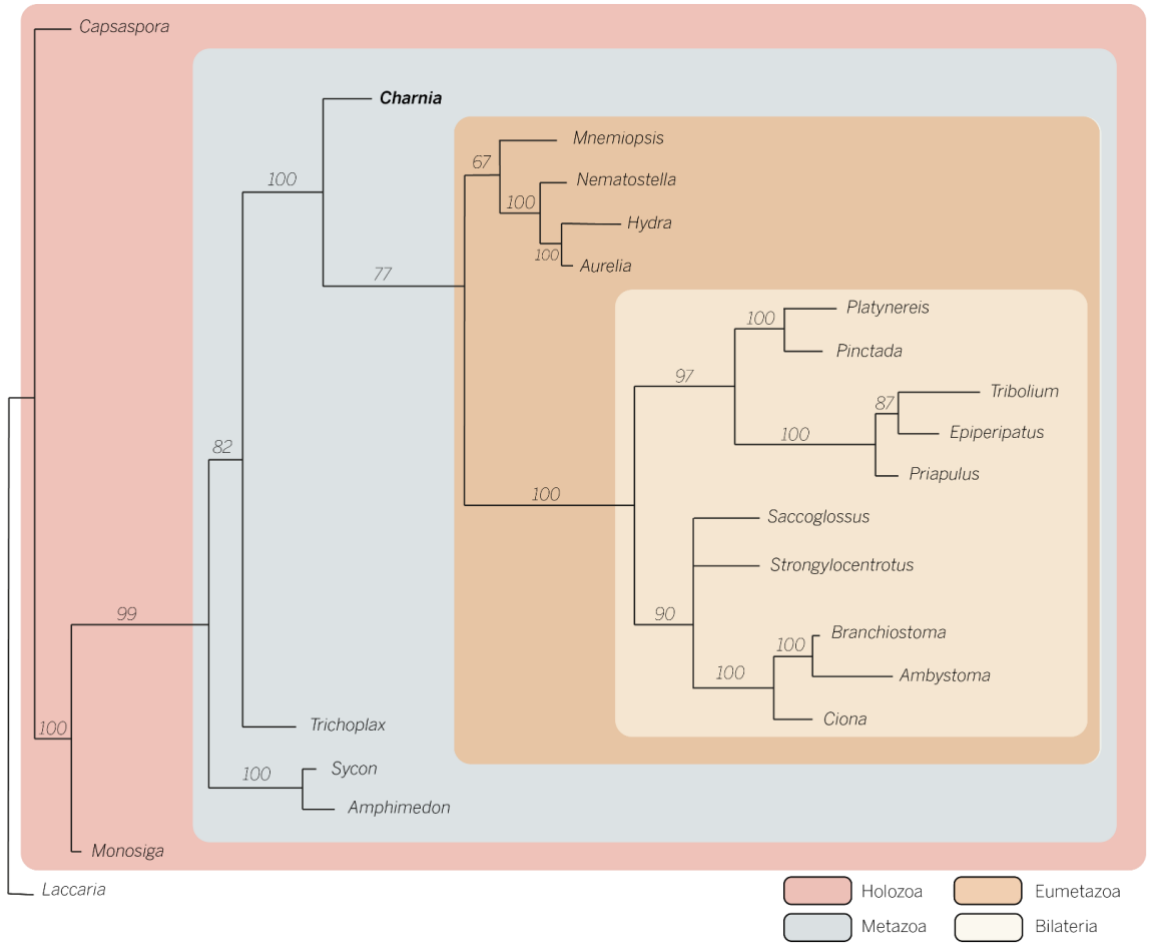


**Figure 5.8:** Phylogram from Bayesian analysis with best supported tree resolving *Charnia masoni* as a stem-group eumetazoan. The results were generated with majority consensus of 7500 trees. Posterior probabilities recorded at nodes.

| Outgroup to *Monosiga* | Hypothesis 1 | Hypothesis 2 | Bayes Factor |
|---|---|---|---|
| M,P | -550.11 | -542.92 | -7.19 |
| M,T | -550.11 | -542.16 | -7.95 |
| M,E | -550.11 | -532.68 | -17.43 |
| M,C | -550.11 | -534.91 | -15.20 |
| M,Cn | -550.11 | -536.44 | -13.67 |
| M,B | -550.11 | -536.83 | -13.28 |
| M,Pr | -550.11 | -551.45 | 1.34 |
| M,D | -550.11 | -550.90 | 0.79 |

| Outgroup to *Porifera* | Hypothesis 1 | Hypothesis 2 | Bayes Factor |
|---|---|---|---|
| P,T | -542.92 | -542.16 | -0.76 |
| P,E | -542.92 | -532.68 | -10.24 |
| P,C | -542.92 | -534.91 | -8.01 |
| P,Cn | -542.92 | -536.44 | -6.48 |
| P,B | -542.92 | -536.83 | -6.09 |
| P,Pr | -542.92 | -551.45 | 8.53 |
| P,D | -542.92 | -550.90 | 7.98 |

| Outgroup to *Placozoa* | Hypothesis 1 | Hypothesis 2 | Bayes Factor |
|---|---|---|---|
| T,E | -542.16 | -532.68 | -9.48 |
| T,C | -542.16 | -534.91 | -7.25 |
| T,Cn | -542.16 | -536.44 | -5.72 |
| T,B | -542.16 | -536.83 | -5.33 |
| T,Pr | -542.16 | -551.45 | 9.29 |
| T,D | -542.16 | -550.90 | 8.74 |

| Outgroup to *Eumetazoa* | Hypothesis 1 | Hypothesis 2 | Bayes Factor |
|---|---|---|---|
| E,C | -532.68 | -534.91 | 2.23 |
| E,Cn | -532.68 | -536.44 | 3.76 |
| E,B | -532.68 | -536.83 | 4.15 |
| E,Pr | -532.68 | -551.45 | 18.77 |
| E,D | -532.68 | -550.90 | 18.22 |

| Outgroup to *Coelenterata* | Hypothesis 1 | Hypothesis 2 | Bayes Factor |
|---|---|---|---|
| C,Cn | -534.91 | -532.44 | 1.53 |
| C,B | -534.91 | -536.83 | 1.92 |
| C,Pr | -534.91 | -551.45 | 16.54 |
| C,D | -534.91 | -550.90 | 15.99 |

| Outgroup to *Cnidaria* | Hypothesis 1 | Hypothesis 2 | Bayes Factor |
|---|---|---|---|
| Cn,B | -532.44 | -536.83 | 0.39 |
| Cn,Pr | -532.44 | -551.45 | 15.01 |
| Cn,D | -532.44 | -550.90 | 14.46 |

Key:
M = Monosiga (Choanoflagellate)
P = Porifera
T = Trichoplax (Placozoa)
E = Eumetazoa
C = Coelenterata
Cn = Cnidaria
B = Bilateria
Pr = Protostomia
D = Deuterostomia

**Table 5.2:** Bayes Factors for competing phylogenetic hypotheses relating to the phylogenetic position of *Charnia masoni*. Bayes Factors were produced by creating backbone constraint trees, and performing a stepping-stone analysis (Xie *et al.* 2010) to give the marginal likelihoods (shown under 'Hypothesis 1' and 'Hypothesis 2'). Hypothesis one is the first named phylogenetic position and hypothesis two is the second named phylogenetic position in each case. Each tested position is a sister relationship to the named group.

My analysis provides strong support for a crown-group metazoan affinity for rangeomorphs. *Charnia masoni* appears in the fossil record at ~571 million years ago and, as such, rangeomorphs constitute the oldest confirmed crown-group animals. While older fossils have previously been described as belonging to the crown-group Metazoa, these all remain contentious. Fossil embryos from the Doushantuo Formation in China have previously been interpreted as the remains of ancient animals (e.g. Xiao *et al.* 1998, Liu *et al.* 2010), but a number of these claims have been challenged (e.g. Hultgren *et al.* 2011; Cunningham *et al.* 2015). The Lantian biota, also described from China (e.g. Wan *et al.* 2016), bears fossils that are sometimes described as animals (e.g. *Lantianella*; Van Iten *et al.* 2013), but these interpretations have also been questioned (e.g. Wan *et al.* 2016). Rangeomorphs were a hugely successful group in the late Neoproterozoic oceans. They diversified rapidly after their first recorded appearance (e.g. Liu *et al.* 2012), in what represents the earliest known animal adaptive radiation (Hoyal Cuthill and Conway Morris 2014), with perhaps multiple rangeomorph lineages invading similar ecospace.

The data presented in this chapter has further implications for our interpretations of rangeomorph taxonomy. If the second order branch is confirmed as the fundamental repeated unit in other rangeomorph taxa, future schemes should look to characterise the variation in anatomy in this branching order for use in an emended defintion of clade Rangeomorpha. Furthermore, consideration of the homology scheme presented here is important for generic-level rangeomorph taxonomy, and the identification of different branching orders. If we consider the number and anatomies of lower branching orders to be of taxonomic significance, we must ensure that we are comparing homolgous structures. If the main frond of *Charnia masoni* is homologous to a single ‘first order branch’ on *Avalofractus abaculus*, then the term first order branch is not consistent across these taxa. Perhaps what have traditionally been called ‘first order branches’ on *A. abaculus* should be referred to as folia, as with the multiple frondose elements on multerminal rangeomorphs.

## 5.5 Conclusions:

This study provides evidence for greater morphogenetic complexity and morphological disparity than has been assumed in rangeomorphs, as well as compiling a new homology scheme that draws parallels between taxa previously considered disparate. I marshal phylogenetic statistical support for *Charnia masoni* as a crown-group metazoan using a conservative phylogenetic approach. This study represents the most expansive investigation of the biology and affinities of a rangeomorph taxon to date, and highlights the considerable advances that can be made with such an approach.

Rangeomorphs, with their unique serially branched body plan, increase the anatomical repertoire of the animals. The radiation of these crown-group animals in the Neoproterozoic oceans acts to presage the radiation of the crown-group bilaterians in the early Cambrian, and supports hypotheses framing the 'Cambrian Explosion' as only one of a series of metazoan radiations from around this time (e.g. Tarhan *et al.* 2018; Wood *et al.* 2019).

# Chapter 6:

## Concluding remarks:

**Author contributions:** This chapter was designed and developed by F.S.D. F.S.D produced the first draft of this chapter. A.G.L provided feedback on this chapter. F.S.D contributed ~97% of the work presented in this chapter.

# Summary:

In this thesis, I have presented new lines of evidence with which we may constrain the affinities of some of the most enigmatic members of the Ediacaran Macrobiota; the rangeomorphs and arboremorphs. I find that current data supports the interpretation of these forms as crown-group animals. In this final chapter, the implications of these results are explored for the study of the evolution of development, and patterns in animal evolution more generally. Finally, future research directions leading from this thesis are outlined.

## 6.1 Ediacaran animals

*(a) Phylogentic Affinities*

A metazoan affinity for many members of the Ediacaran Macrobiota has long been hypothesised, but the unusual anatomies of Ediacaran macrofossils have meant that confirming an animal identity for many of these organisms has proven difficult. Data presented in this thesis provide support for the animal affinities of certain key Ediacaran taxa and, as such, inform our understanding of the evolution of early animals. Resolving *Charnia masoni* as a crown-group metazoan, and *Arborea arborea* as a total-group eumetazoan represents a significant improvement on the potential phylogenetic placements open for these taxa at the beginning of this project.

In Chapter two, I provided a new synthesis of anatomical and developmental data concerning three Ediacaran macroscopic morphogroups; the rangeomorphs, the dickinsoniomorphs and the erniettomorphs. Using this information, I resolved the rangeomorphs and dickinsoniomorphs as total-group metazoans, but find that there is not enough information to constrain the affinities of the erniettomorphs with any degree of certainty.

In Chapter three, I demonstrated a total-group eumetazoan affinity for *Arborea arbroea*, and present evidence that may suggest this organism was able to hydrostatically regulate its holdfast. Moreover, I presented the first evidence for *Arborea* outside South Australia. New specimens, described herein, from the classic Avalonian deep-water turbiditic Charnwood Forest deposits are dated at between 557 and 562 million years old (Wilby *et al.,* 2011). These specimens extend the known stratigraphic range of *Arborea* by up to seven million years, and extend the known environmental tolerances of the organism. These data indicate that eumetazoans were geographically widespread by this time. Finally, I concluded that the anatomy of *Arborea* is consistent with a modular and perhaps colonial grade of organisation. Coloniality is well-documented as having evolved independently in many animal groups and so, of itself, serves no clear phylogenetic purpose. However, if this interpretation is correct, this would suggest this area of metazoan morphospace was occupied before the Cambrian

Period (see also Dewel 2000). This contradicts recent work which concludes colonial eumetazoans do not appear in the fossil record until the Ordovician (Landing *et al.* 2018).

In Chapters four and five, I provided a new model of the anatomy of *Charnia masoni*, and demonstrated a crown-group metazoan affinity for this taxon and, presumably, other rangeomorphs. *Charnia masoni* is present from ~571 million years ago and exhibits an anatomy that is unknown from extant animal groups. *Charnia* therefore expands the described morphological disparity of animals. Indeed, rangeomorph disparity, typically described by the number of terminal tips the fronds possess, may misrepresent bodyplan homologies between different taxa. I conclude that we may need to revisit studies that unite groups of rangeomorphs together based on their number of distal tips (e.g. Dececchi *et al.* 2017, 2018), and suggest that multiple rangeomorph lineages may have invaded similar positions in ecospace.

*(b) Morphogenesis*

I revealed a highly-constrained pattern of development in *Charnia* that undermines previous hypotheses concerning the evolution and radiation of the rangeomorphs, which are reliant on a presumed morphogenetic simplicity or phenotypic plasticity (Hoyal Cuthill and Conway Morris 2014, 2017). I suggested that the branching architecture of *Charnia* was governed primarily by internal factors, not by the external environmental responsiveness that governs the final morphology of many extant branching organisms, which may have implications for limiting ecophenotypic variation past simple morphometric change. My data reveal that the frond of *Charnia* is generated by the specification of successive lateral branching axes in a sympodial fashion, with new second order branches being generated from an apical growing tip in a given first order branch. In contrast, the developmental pattern observed in *Arborea* sees tubular structures grow towards the apex within a central stalk, before erupting from the lateral margin of the stalk and forming a single lateral branch (including the 'pod' and the individual units). Each unit is independent of the others, being connected only via a tubular connection to the basal tubular structure. In the smallest known specimen, lateral branches are observed only as bulbous projections. This raises the possibility that lateral branches

'open' to reveal individual units at a later developmental stage, although further specimens of a similar size are required to confirm or refute this suggestion.

This new morphogenetic information does not reveal any characters that would favour a close relationship between these two taxa over more distant phylogenetic kinship. Indeed, the distinct patterns of branching morphogenesis may suggest a more distant relationship between these two taxa. This is of note because both *Arborea* and *Charnia* possess an ostensibly similar frondose anatomy. Ediacaran frondose taxa have historically been a taxonomic conundrum, with some authors considering *Charniodiscus* a close ally of the rangeomorphs (e.g. Antcliffe and Brasier 2009), some considering *Charniodiscus* an arboreomorph (which are distinct from the rangeomorphs), and more recently various species of *Charniodiscus* being syonymised within the taxon *Arborea*, as discussed in Chapters one and three. It may be the case that phylogenetically disparate animal groups converged on a frondose anatomy in the late Neoproterozoic (see description of these organisms in Xiao and Laflamme 2009). Many other Ediacaran macrofossils (of unknown affinity) display a frondose anatomy (e.g. *Swartpuntia germsii, Bomakellia kelleri* or *Pambikalbae hasenohrae*) and are either considered to fall within different Ediacaran morphogroups, or not within any described morphogroups as currently defined (SOM of Erwin *et al.* 2011). This may suggest that a frondose form was better adapted to certain late Neoproterozoic environments than alternative anatomies, although the reasons underlying this preference is unknown. It has been proposed that the frondose habit was occupied in order to facilitate nutrient extraction from the water column (e.g. Laflamme and Narbonne 2008b; Laflamme *et al.,* 2009).

It is interesting to note that extant animal groups with a frondose anatomy are generally colonial (e.g. cnidarians or bryozoans) in organisation, while branched sponges may sometimes assume a quasi-frondose form (e.g. the carnivorous *Chondrocladia gigantea*). This may imply a similar mode of organisation for some Ediacaran frondose organisms, for example the rangeomorphs or *Arborea arborea* (as further discussed in Chapter three).

*(c) Other Ediacaran animals?*

I find that *Charnia* is more derived than the sponges (Fig. 5.8), and consider sponges to be the earliest diverging animal group (e.g. Simion *et al.* 2017; Feuda *et al.* 2017). These data provide further corroborative evidence to suggest that Precambrian sponges were present, and that the lack of compelling Precambrian sponge bodyfossils is an artefact; the result of either preservational bias, or our current inability to recognise fossil sponges (either crown group or stem group taxa) from this time. Reports of biomarkers present in sponges (most famously 24-isopropylcholestane, Love *et al.* 2009, but also see Zumberge *et al.* 2018) from the Neoproterozoic should be treated with caution (e.g. Antcliffe, Callow and Brasier 2014; Nettersheim *et al.* 2019), but remain a maximum constraint on the appearance of sponges.

Furthermore, instances of Ediacaran cnidarian-grade organisms from the time of the rangeomorphs, although rare, are reasonably compelling (e.g. Liu *et al.* 2010; Liu *et al.* 2014). Together, these data and data presented in this thesis support the idea of a diverse metazoan fauna in the late Ediacaran Period.

## 6.2 Ediacaran Macrofossils and the evolution of development

Work produced for this thesis has elucidated the developmental pattern of *Charnia masoni* and *Arborea arborea,* and an additional study published in 2018 has provided insight into the developmental biology of another rangeomorph; *Hylaecullulus fordii* (Kenchington *et al.,* 2018). Further work concerning the dickinsiomorphs has provided a model of growth for the iconic *Dickinsonia* (Chapter two; Hoekzema *et al.* 2017, though see Evans *et al.* 2017), while some progress has been made in understanding growth in the rare dickinsoniomorph taxon *Andiva ivantsovi* (Chapter two; Evans *et al.* 2018). Together, these advances (in conjunction with other studies, e.g. Bobrovskiy *et al.* 2018) allow us to make inference about the evolution of metazoan developmental dynamics. However, in the absence of a unique phylogenetic placement for any of these named taxa, these conclusions remain preliminary.

All known rangeomorphs display a form of radial symmetry, but the form they bear is variable. *C. masoni* and perhaps *Fractofusus* exhibit biradial symmetry, *Rangea schneiderhoehni* exhibits hexaradial symmetry, and other forms like *Bradgatia* possess an unknown variant of radial symmetry. There is some debate concerning whether rangeomorph branching is offset at all branching orders. Flude and Narbonne (2008) describe *Bradgatia* as occasionally expressing bilaterally symmetrical sides. Recently, the publication of the draft genome of the hydrozoan *Clytia hemisphaerica* has been used to suggest that the plesiomorphic symmetry state for the Medusozoa was not bilateral (Leclère *et al.* 2019). This is on the basis that the HOX cluster found in the anthozoan *Nematostella vectensis* is missing in *C. hemisphaerica* and *Hydra magnipapillata.* However, whether or not the results of He *et al.* (2018), do in fact indicate homology between these two axes, or whether this is the result of gene co-option (discussed below) remains undertermined. That said, these data imply a secondary acquisition of bilaterality in a select few hydrozoan groups, the molecular underpinnings of which remain unknown. The potential evidence for bilateral symmetry in higher order branches in rangeomorph units discussed in Chapter two may then also represent an independent cooption of the bilateral state, and highlights the capacity for rangeomorphs to experiment with forms of symmetry.

This view stands opposed to the classical ideas of promorphology (Beklemishev 1964), which propose a linear evolutionary trajectory from radially symmetrical forms to bilateral forms, as applied to Ediacaran taxa (e.g. Fedonkin 1985). At the time Fedonkin was writing, the forms of symmetry exhibited by various Ediacaran taxa were considered to represent the plesiomorphic or early diverging conditions for extant groups, which corroborated their description as being antecedents to extant sub-phylum clades. The re-description of some of these taxa as belonging to more inclusive clades suggests a more complex evolution of patterns of symmetry in the animals, and perhaps a greater variation in symmetry amongst early metazoan ancestors, rather than a step-wise acquisition of symmetry states.

Whether metazoan body axes are homologous or not is debated; are the oral-aboral and anterio-posterior axes homologous, or the directive and dorso-ventral axes? There appears to be some support for axis homology between some non-bilaterian and bilaterian axes, for example through similar HOX patterning across the directive and dorso-ventral axes (He *et al.*

2018), but these data could equally be interpreted as a result of co-option (e.g. Setton *et al.* 2018).

I conclude that there was a disparity of animal body plans present by the late Ediacaran. *Charnia masoni,* and all other examined rangeomorphs, do not exhibit front-back differentiation, but *Arborea arborea* does. This indicates that late Ediacaran frondose forms possessed a variation in their number of principal body axes. The relationship between these different axes and those of extant taxa remains, in the absence of a more refined phylogenetic placement, unknown, and rationalising between these anatomies will require a more refined understanding of non-bilaterian axis homologies. I can, however, reject the hypothesis that Ediacaran fronds including *C. masoni* and *A. arborea* indicate a more complex stem-lineage to the Metazoa than perhaps suggested by our understanding of the inter-relationships of extant groups, with Choanoflagellata as sister to a monophyletic Porifera (e.g. Fueda *et al.* 2017).

Before the true significance of these fossils in elucidating the evolution of development can be disentangled, we must establish a broader suite of non-bilaterian model systems and genomes with which to investigate questions concerning the evolution of key metazoan characters and the inter-relationships of non-bilaterian metazoans. The first non-bilaterian metazoan genome was only published in 2007 (*Nematostella vectensis*; Putnam *et al.* 2007) and while genomes are now available for representatives of all non-bilaterian phyla, they remain markedly undersampled. Until recently, only one placozoan species was known (*Trichoplax adherens*), but recently a greater number of placozoan genomes have been published, and a new genus erected (Eitel *et al.* 2018; Laumer *et al.* 2018). These new studies support a greater diversity in placozoan genomes than previously considered. Laumer *et al.* 2018 provide support for a clade of Placozoa + Cnidaria, whereas previous analyses placed Placozoa as sister to the Planulozoa (Cnidaria + Bilateria) (e.g. Simion *et al.* 2017; Feuda *et al.* 2017). This recent work shows how the undersampling of non-bilaterian metazoans may be obscuring the inter-relationships of these early-diverging clades. Another ongoing debate concerns the root of the animal tree; did the morphologically comparatively 'simple' poriferans emerge first, or did the ctenophores? Recent work has suggested that with greater taxon sampling (Simion *et al.* 2017) and improved modelling of compositional heterogeneity

(Feuda *et al.* 2017) the sponges are consistently resolved as the earliest diverging animal group. This is the phylogenetic hypothesis preferred by the author in this thesis.

At a developmental genetic level, it is known that *Wnt* signalling plays a role in specifying the primary body axis of cnidarians and bilaterians, but the role of *Wnt* in specifying the primary body axis of the sponges remains contentious. Some sponges, for example the Glass sponges, do not express *Wnt* proteins at all (Schenkelaars *et al.* 2017; Renard *et al.* 2018), while others appear to express diverse *Wnt* complements (e.g. Boriensko *et al.* 2016). Some have argued that *Wnt* proteins are not used to signal between structures in freshwater sponges (Windsor Reid *et al.* 2018). Myxozoa, a group of derived parasitic cnidarians, also appear to have lost *Wnt* proteins (Chang *et al.* 2015), suggesting that the role of Wnts in specifying the cnidarian primary body is not straightforward. In placozoans, recent work has gone some way to suggest homology between the radial axis of placozoans and the oral-aboral axis of cnidarians (DuBuc *et al.* 2019), but a current inability to observe the embryogenesis of placozoan taxa hampers more conclusive statements. Once debates like these are resolved by greater study and sampling of extant non-bilaterian metazoans, fossils of the Ediacaran Macrobiota – if a unique phylogenetic placement can be resolved – will inform the evolution of these characters.

## 6.3 Future directions

The taxa on which this thesis is based represent icons of the Ediacaran Macrobiota. This in itself is not necessarily a problem for the studies presented here but raises two points concerning the future of Ediacaran palaeobiology. While there are a large number of described Ediacaran macrofossil taxa, only a handful of these organisms (e.g. *Charnia* or *Dickinsonia*) have previously attracted significant research attention. Historically, these fossils were considered monophyletic (e.g. Seilacher 1989; Buss and Seilacher 1994; Hoyal Cuthill and Han 2018), perhaps justifying the study of only a few of these taxa as representative of biology and diversity of the whole group. However, at the time of writing, consensus tends towards a polyphyletic assemblage (e.g. Wood *et al.* 2019) throwing the broader applicability of findings from these 'iconic' taxa into doubt. Organisms like *Tribrachidium heraldicum,*

*Rugoconites enigmaticus* or *Inaria karli* bear little morphological similarity to other Ediacaran forms, while many other frondose taxa (e.g. *Parviscopa bonavistensis*) cannot readily be compared to the rangeomorphs or arboreomorphs.

Even relatively well-known Ediacaran groups remain understudied as compared to *Charnia masoni* and *Dickinsonia.* Currently, there is no positive evidence to tie the erniettomorphs to any particular phylogenetic group, but a wealth of specimens (e.g. Grazhdankin and Seilacher 2002; Elliott *et al.* 2015; Smith *et al.* 2017) mean that they are the obvious next target for the integrated developmental and phylogenetic approach advocated here. The erniettomorphs are the youngest of the soft-bodied Macrobiota, only disappearing from the rock record at or after the Precambrian-Cambrian boundary (Jensen, Gehling and Droser 1998; Smith *et al.* 2017) and so may provide insight into the Ediacaran – Cambrian transition that is not provided by other Macrobiota taxa.

That is not to say that research interest in rangeomorph taxa is misplaced; there remains a huge body of work left to be done. The taxonomy of both the rangeomorphs and the arboreomorphs remains in flux, and this hampers the production of new morphogroup level diagnoses. Rangeomorphs are the best studied Ediacaran morphogroup, and represent one of the most ancient adaptive radiations that the fossil record preserves, and, certainly, the oldest animal adaptive radiation. Understanding how this group diversified is then of importance in understanding whether the processes underlying evolutionary patterns have remained uniform over time, or have changed. Developmental analysis in other taxa, for example *Fractofusus*, would permit testing of the hypotheses advocated in this thesis (e.g. indeterminate growth) while reconsideration of what constitutes a homologous structure may result in changes to the taxonomic nomenclature and its implementation. Together, these will result in a better understanding of rangeomorph interrelationships, crucial if we are to understand the radiation of this group.

Further inclusion of Ediacaran taxa in morphological phylogenetic analyses where chosen characters are justified and follow best practise, will no doubt prove informative on the affinities of these groups to extant lineages. Previously proposed hypotheses of affinity, seemingly based on equivocal evidence (e.g. Jenkins and Nedin 2007; Fedonkin and Ivantsov

2007), may be reassessed. A prime example is the association of the frondose *Pambikalbae hasenohrae* to the hydrozoan cnidarians (Jenkins and Nedin 2007) in the absence of any hydrozoan diagnostic characters (Daly *et al.* 2007). A potential obstacle remains our inadequate understanding of phenotypic evolution; the simplistic nature of the Mk model is unlikely to model the evolution of character states accurately. However, these model-based approaches, when implemented in a Bayesian phylogenetic framework, still appear to outcompete traditional parsimony-based approaches (e.g. O'Reilly *et al.* 2016; O'Reilly *et al.* 2018; Puttick *et al.* 2019).

Equally, in order to establish the role these fossils may play in improving our understanding of early animal evolution we must expand our sampling of extant non-bilaterian metazoans. Understanding metazoan developmental synapomorphies is crucial if we are to rationalise between these strange Ediacaran fossils and the members of living groups because they shape our interpretative framework; for instance, if all metazoan primary body axes are homologous, then we can consider the evolution of metamerism across this axis including Ediacaran fossils. To this end, the establishment of functional genomics in non-bilaterian model systems will be crucial (e.g. the establishment of Crispr in *Nematostella vectensis*; He *et al.* 2018). Similarly, open questions concerning the role of *Wnt* signalling (discussed above) in early diverging metazoan clades such as the sponges are becoming increasingly tractable with the establishment of functional genomics in these groups. Whether or not the demosponges possess true epithelia is another crucial question.

In addressing these open phylogenetic questions, I hope we may better understand the biotic landscape during the Ediacaran Period. This will allow workers to test hypotheses concerning the evolution of form, for example the multiple independent(?) originations of a frondose anatomy; the exploration of rare radial symmetry states (e.g. the triradialomorphs; Hall *et al.* 2018); the acquisition of multiple body axes, or the advent of metazoan locomotion (Ivantsov and Malakovskaya 2002; Liu *et al.* 2010). In the last twenty years, huge strides have been made in understanding the members of the Ediacaran Macrobiota, and the environments they inhabited. We must begin work in earnest on non-model Ediacaran groups, and incorporate them into macroevolutionary studies alongside Phanerozoic taxa. Future workers should strive to view the fossil records of the Ediacaran and Cambrian Periods as a succession,

recording a sequence of successive metazoan radiations, rather than time slices recording independent phenomena. This will allow us to assess trends in the evolution of morphological disparity, ecospace and niche occupancy, and elucidate the true significance of the Ediacaran Macrobiota in documenting the rise of the animals.

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# Appendix 1:

**Chapter three specimen list:**

Specimens from South Australia (all from South Australia Museum, or remain in field in South Australia, as indicated in text)

**SAM P19690a** – articulated specimen with holdfast

**SAM P19690b**

**SAM P493641a**

**SAM P493641b**

**SAM P40858a-h** – incomplete articulated specimen with holdfast

**SAM P40306** – holdfast

**SAM P48727** – articulated specimen

**SAM P47800**

**SAM P47799**

**SAM P14213**

**SAM P12894**

**SAM P40952**

**SAM P13787**

**SAM P12892**

**SAM P42686**

**SAM P14212**

**SAM P12890**

**SAM P14214**

**SAM P13800**

**SAM P13801a**

**SAM P40769**

**SAM P40832**

**SAM P40772**

**SAM P40785** – smallest studied articulated specimen

**SAM P34499**

**SAM P40369**

**SAM P40572**

**SAM P40787**

**SAM P40792**

**SAM P40825**

**SAM P40786**

**SAM P40773**

**SAM P40797**

**SAM P40775**

**SAM P40771**

**SAM P45405** – holdfast

**SAM P40332** – holdfast and stalk

**SAM P35704b**

**SAM P49416**

**SAM P35702a**

**SAM P35702b**

**SAM P35702c**

**SAM P51201**

**SAM P51200**

**SAM P40309** – holdfast

**‘52’** – holdfast and stalk

**P12888** – holdfast

**Field specimens from Bunyeroo Gorge**

**Large unaccessioned field specimens**

Specimens from Charnwood Forest, UK. All specimens housed at the British Geological Survey.

**GSM 105960** – articulated specimen

**GSM 106024**

**‘cast 629’**

## Chapter four specimen list:

Specimens from Charnwood Forest, UK. All specimens housed at the British Geological Survey.

**GSM 105978** – specimen with a basal extension

**GSM 106040** – specimen with a basal extension

**LEIUG 2328** – specimen with a basal extension

**GSM 106078** – specimen with a basal extension

**GSM 105989** – specimen with a basal extension

**GSM 105979** – specimen with a basal extension

**GSM 105997** – specimen with a basal extension

**GSM 105972** – specimen with a basal extension

**GSM 105974** – specimen with a basal extension

**GSM 105966** – specimen without a basal extension

**GSM 106084** – specimen without a basal extension

**GSM 105994** – specimen without a basal extension

***Unlabelled BGS cast*** – specimen without a basal extension

**GSM 105877** – specimen without a basal extension

**GSM 105993** – specimen without a basal extension

**GSM 105996** – specimen without a basal extension

**GSM 105990** – specimen without a basal extension

**GSM 105987** – specimen without a basal extension

**GSM 106082** – specimen without a basal extension

**LEIUG 2328** – specimen with lateral branches

**GSM 105993** – specimen with lateral branches

**GSM 105972** – specimen with lateral branches

**GSM 105997** – specimen with lateral branches

**GSM 105966** – specimen with lateral branches

**GSM 105978** – specimen with lateral branches

**GSM 105996** – specimen with lateral branches

**GSM 105990** – specimen with lateral branches

***Unlabelled BGS cast*** – specimen without lateral branches

**GSM 106078** – specimen without lateral branches

**GSM 105979** – specimen without lateral branches

**GSM 106040** – specimen without lateral branches

**GSM 105989** – specimen without lateral branches

**GSM 105994** – specimen without lateral branches

**GSM 105987** – specimen without lateral branches

**GSM 106084** – specimen without lateral branches

**GSM 106082** – specimen without lateral branches

**GSM 105983** – additional specimen used to examine morphology

**GSM 105973** – additional specimen used to examine morphology

**GSM 105982** – additional specimen used to examine morphology

**GSM 105980** – additional specimen used to examine morphology

**GSM 105988** – additional specimen used to examine morphology

**GSM 105985** – additional specimen used to examine morphology

**GSM 105975** – additional specimen used to examine morphology

**GSM 105977** – additional specimen used to examine morphology

**GSM 105984** – additional specimen used to examine morphology

**GSM 106079** – additional specimen used to examine morphology

**GSM 105995** – additional specimen used to examine morphology

**GSM 106081** – additional specimen used to examine morphology

**GSM 106086** – additional specimen used to examine morphology

**GSM 106080** – additional specimen used to examine morphology

**GSM 105992** – additional specimen used to examine morphology

**GSM 105971** – additional specimen used to examine morphology

**GSM 105986** – additional specimen used to examine morphology

**GSM 105981** – additional specimen used to examine morphology

**GSM 105976** – additional specimen used to examine morphology

**GSM 106039** – additional specimen used to examine morphology

**GSM 106000** – additional specimen used to examine morphology

**GSM 105959** – additional specimen used to examine morphology

**GSM 106001** – additional specimen used to examine morphology

Specimens collected in Newfoundland during 2016 – 2017 field seasons. Specimens accessioned at the Sedgwick Museum, Cambridge. Unaccessioned specimens (beginning N17) housed at the University of Cambridge

**X. 5097.11** – specimen with a connecting region

**X. 50297.7** – specimen with a connecting region

**X. 50297.10** – specimen with a connecting region

**X. 50297.4** – specimen with a connecting region

**X. 50297.5** – specimen with a connecting region

**X. 50297.2** – specimen with a connecting region

**X. 50297.3** – specimen with a connecting region

**X. 50297.6** – specimen with a connecting region

**X.50297.9** – specimen with a connecting region

**N17 – LC6 – 03** – cast with *C. masoni* with connecting region

**N17 – LC6 – 05** – cast with *C. masoni* with connecting region

**N17 – LC6 – 06** – cast with *C. masoni* with connecting region

**N17 – LC6 – 07** – cast with *C. masoni* with connecting region

**N17 – LC6 – 08** – cast with *C. masoni* with connecting region

**N17 – LC6 – 09** – cast with *C. masoni* with connecting region

*Other specimens are not cast and remain in the field*

**X. 50297.1** – specimen without a connecting region

*Other specimen is not cast and remains in the field*

Specimen accessioned at the Oxford University Museum of Natural History.

**OUMNH ÁT.429/p** – additional specimen used to examine morphology

Specimens held at the Palaeontological Institute, Moscow (fragmentary remains)

**PIN 3993 – 7018** – additional specimen used to examine morphology

**PIN 3993 – 7023** – additional specimen used to examine morphology

**PIN 3993 – 7025** – additional specimen used to examine morphology

**PIN 3993 – 7020** – additional specimen used to examine morphology

## Chapter five specimen list:

**GSM 105994** – specimen used in Fig. 5.4A-D

**GSM 105983** – specimen used in Fig. 5.4A-D

**GSM CL21803** – specimen used in Fig. 5.4A-D

**GSM 106079** – specimen used in Fig. 5.4A-D

**LEIUG 2328** – specimen used in Fig. 5.4A-D

**GSM 105873** – specimen used in Fig. 5.4A&C *only*

**GSM 103987** – specimen used in Fig. 5.4A-D

**GSM 105982** – specimen used in Fig. 5.4A-D

**GSM 1065992** – specimen used in Fig 5.4A-D

**GSM 106078** – specimen used in Fig 5.4A-D

**GSM 105944** – specimen used for growth analyses

**GSM 106084** – specimen used for growth analyses

**GSM 105989** – specimen used for growth analyses

**GSM 105997** – specimen used for growth analyses

**LEIUG 2328** – specimen used for growth analyses

**GSM 105873** – specimen used for growth analyses

**GSM 106160** – specimen used to quantify number of second order branches proximal to differentiating first order branch

**GSM 105873** – specimen used to quantify number of second order branches proximal to differentiating first order branch

**GSM 105989** – specimen used to quantify number of second order branches proximal to differentiating first order branch

**GSM 105877** – specimen used to quantify number of second order branches proximal to differentiating first order branch

**GSM 105977** – specimen used to quantify number of second order branches proximal to differentiating first order branch

**GSM 105873** – specimen used to quantify number of second order branches proximal to differentiating first order branch

**GSM 106084** – specimen used to quantify number of second order branches proximal to differentiating first order branch

**CGSM 1079 – 102** – specimen used to quantify number of second order branches proximal to differentiating first order branch

**CGSM 2079 – 100** – specimen used to quantify number of second order branches proximal to differentiating first order branch

**CGSM 2079 – 100** – specimen used for tomographic analyses (CT scan)

**CGSM 2079 – 101** – specimen used for tomographic analyses (CT scan)

**CGSM 2079 – 102** – specimen used for tomographic analyses (CT scan)

**CGSM 2079 – 103** – specimen used for tomographic analyses (CT scan)

**CGSM 2029 – 104** – specimen used for tomographic analyses (CT scan)

**CGSM 2079 – 105** – specimen used for tomographic analyses (synchrotron scan)

# Appendix 2:

## Phylogenetic analyses:

Character list:

Characters were coded from references 1-3, except where noted. Multistate characters where the absence of a given character was a state are treated as multiple characters (with contingencies noted herein) in order to weight presence and absence equally.

Unless otherwise noted, characters are binary, with 0 representing character absence, and 1 representing character presence.

*Character list:*

1. Hyphae [1] *contingent on 4.*
2. Sporocarp [1] *contingent on 4.*
3. Active motility (at any life stage) [holozoan motility in 3]
4. Multicellularity [2]
5. Amoeboid stage [3]
6. Presence of cyst for part of life cycle [3]
7. Somatic cell differentiation [2] *contingent on 4.*
8. Meiosis [2,3]
9. Diploid zygote [2,3]
10. Spermatogenesis [2]
11. Oogenesis [2]
12. Impermeable cell-cell connections [2] *contingent on 4.*
13. Extracellular matrix [2] *contingent on 4.*

14. Aquiferous system [2] *contingent on 4.*

15. Mesohyl [2] *contingent on 4.*

16. Pinacocytes [2]

17. Choanocytes [2]

18. Mineral spicules [2] *contingent on 4.*

19. Mineral spicule type [2] 1 calcareous, 2 siliceous

20. True spongin [2]

21. Archeocytes [4]

22. Organ bearing. A persistent group of tissues in a specific location that perform a specific function. An entire organism cannot represent a single organ. *New character* [lack of organs in *Trichoplax:* 5]*. contingent on 4.*

23. Sensory organs [adapted from 2]. A persistent group of sensory cells in a specific location that perform a specific function. An entire organism cannot represent a single organ. This would include a nerve net and a CNS, for example. *Contingent on 4.*

24. Polarity. Whether an organism possesses a primary body axis. We exclude cellular polarity here. *New character*. *Contingent on 4.*

25. Polarity type. The number of primary body axes an organism possesses. We exclude cellular polarity here. *New character*. *Contingent on 4.*

26. Body symmetry. We exclude cellular symmetry [6]. *Contingent on 4.*

27. Radial body symmetry. 1 – radial, 2 – biradial [2, 5]. *Contingent on 4, 26.*

28. Bilateral body symmetry [2, 5]. *Contingent on 4, 26.*

29. Body constructed of repeated multiply branching units. *New character. Contingent on 4.*

30. Epithelium [2] *Contingent on 4.*

31. Differentiation of two epithelial layers [2] *Contingent on 4, 31.*

32. Epithelial linking of cells by zonulae adherents [2] *Contingent on 4, 31.*

33. External digestive surface [5] *Contingent on 4.*

34. Intermediate body layer populated by fiber cells [5] *Contingent on 4.*

35. Crystal cells [5]

36. Endoderm + Ectoderm [2] *Contingent on 4.*

37. Localised gonads [2] *Contingent on 4.*

38. Gastrulation [8] *Contingent on 4.*

39. Gut cavity with endodermal lining [2] *Contingent on 4, 36*

40. Gut state [9, 10] 1- blind gut, 2 – gut with paired anal pores, 3 – through gut with anus. *Contingent on 4, 36, 39*

41. Muscle cells [2] *Contingent on 4, 36*

42. Muscle tissue type. 1 - epithelial muscle cells, 2 - outer circular and inner longitudinal muscles in body wall, 3 – segmented musculature developed from rows of mesodermal pockets from archenteron [2,11] *Contingent on 4, 36, 41.*

43. Nerve cells with chemical synapses [11] *Contingent on 4.*

44. Nerve cells organised into ganglia [11] *Contingent on 4, 22, 23, 36, 43.*

45. Coelenteron [2, 7] *Contingent on 4, 38, 39.*

46. Synapses with acetylcholine [7] *Contingent on 4, 36, 43*

47. Monociliated sensory cells [2]

48. Gap junctions [2] *Contingent on 4.*

49. Mesoglea (which plays a role in a hydrostatic skeleton) [2] *Contingent on 4.*

50. Polypoid stage [2] *Contingent on 4.*

51. Tentacles [2] *Contingent on 4, 36*

52. Cnidae (as products of cnidocytes or cnidoblasts) [2]

53. Cnidocil apparatus [2] *Contingent on 52*

54. Gastric filaments (which secrete gastric enzymes) protruding into stomach [2] *Contingent on 4, 36, 38, 39*

55. Rhopalia [2] *Contingent on 4.*

56. Microbasic eurystele [2] *Contingent on 10.*

57. Septa in polyp [2] *Contingent on 4, 36, 50.*

58. Velum (projecting in from margin of the bell) [12] *Contingent on 4, 36, 50*

59. Coronal muscle (of medusae)[13] *Contingent on 4, 36, 41, 50*

60. Oral-aboral axis *Contingent on 4, 24.*

61. Comb rows [2] *Contingent on 4, 36.*

62. Colloblasts [2]

63. Ephrya larva [2] *Contingent on 4.*

64. Biradial cleavage [2] *Contingent on 4.*

65. Dorsal-ventral axis *Contingent on 4, 24.*

66. Central nervous system [2] *Contingent on 4, 43, 44.*

67. Dorsal nervous system [14] *Contingent on 4, 36, 43, 44, 67*

68. Ventral nervous system [14] *Contingent on 4, 36, 43, 44, 47*

69. Mixocoel circulatory system [14] *Contingent on 4, 36*

70. Ventral longitudinal nerves: paired or secondarily fused [8] *Contingent on 4, 36, 43, 44, 67, 69.*

71. Metanephridia [12] *Contingent on 4, 36*

72. Protonephridia [2] *Contingent on 4, 36.*

73. Archimery [8] *Contingent on 4*

74. Introvert [2] *Contingent on 4, 36*

75. Scalids [2] *Contingent on 4, 36*

76. Flosculi [2] *Contingent on 4, 36*

77. Lorica [2] *Contingent on 4, 36*

78. Coelomate [8] *Contingent on 4, 36, 38, 39.*

79. Coelum state. 1 – schizocoelous, 2 – enterocoelous [8] *Contingent on 4, 36, 38, 39, 77.*

80. Mesoderm [2] *Contingent on 4, 36*

81. Mesoderm derived directly from archenteron [11] *Contingent on 4, 36, 38, 39.*

82. Blastopore [8]

83. Blastopore fate [8]. 1 remains as mouth/anus, 2 becomes mouth, 3 becomes anus. *Contingent on 4, 38.*

84. Trochophore larva [8] *Contingent on 4.*

85. Spiral cleavage [8] *Contingent on 4.*

86. Lophophore [2] *Contingent on 4, 36, 38, 39.*

87. Radula [2] *Contingent on 4, 36*

88. Mantle with mantle groove [2] *Contingent on 4, 36*

89. Setae [2] *Contingent on 4, 36*

90. Chaetae [2] *Contingent on 4, 36*

91. Parapodia [2] *Contingent on 4, 36*

92. Three layered cuticle [2, 11] *Contingent on 4, 36*

93. Body cuticle moulted [2] *Contingent on 4, 36, 92*

94. Digestive gut without cilia [11] *Contingent on 4, 36, 38, 39*

95. Ecdysteroids [2] *Contingent on 4.*

96. Jointed limbs associated with head [2] *Contingent on 4, 36, 80.*

97. Jointed body [2] *Contingent on 4.*

98. Lobopod [2] *Contingent on 4.*

99. Slime papillae [2] *Contingent on 4.*

100. Notochord [2] *Contingent on 4, 80*

101. Stereoblasts [2] *Contingent on 4.*
102. Stereom/stereom reduced to microsclerites [2] *Contingent on 4.*
103. Respiratory trees [2] *Contingent on 4, 36*
104. Madreporite [8] *Contingent on 4.*
105. Stomochord [2] *Contingent on 4, 80*
106. Collor cord [16] *Contingent on 4, 80*
107. Preoral ciliary organ [17] *Contingent on 4, 80*
108. U shaped pharyngeal slits with collagenous skeleton [11] *Contingent on 4, 80*
109. Entodermal chorda dorsalis beneath the neural tube [2] *Contingent on 4, 80, 98*
110. Post-anal tail [2] *Contingent on 4, 80.*
111. Tunica (made of tunicin) [2] *Contingent on 4, 80.*
112. Endostyle [2] *Contingent on 4, 80, 106*
113. Vertebral column [2] *Contingent on 4, 80*
114. Neural crest cells [2] *Contingent on 80*
115. Dermal bone [2] *Contingent on 80.*
116. Perichondral bone [2] *Contingent on 4, 80*
117. Cartilage [2] *Contingent on 4, 80*

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*Character matrix:*

*Charnia 00?1??1??????????0???1?211201??????????0-?-??????????--?-?-?0??????0--?-???000???0?????0000-0--000000?00-000000000?000*
*Laccaria 110?00100000000000-000--00--00---00--0--------000-0-------0----0-0----------0---*
*Capsaspora 001011-00------000-00---00--------0----------0-0-0-------0------0----------0----------0---*
*Monosiga 001000-11------000-00---00--------0----------0-0-0-------0------0----------0----------0---*
*Sycon 00110011111111111110100110--0000000000----0---000-0-------0-0000-0----------0-00---------0----------0---*
*Amphimedon 00110011111111111121100110--00000000000---0---000-0-------0-0000-0----------0-00---------0----------0---*
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*Mnemiopsis 00110011111110000-0011211200110010111121110011110 0-00----011110-00--0----00000--110000-0000-00---0-0--0---------0---*
*Nematostella 00110011111110000 00112111001100101111111110111111100001--100000-00--0----00000--110000-0000-00---0-0--0---------0---*
*Hydra 001100111111100000-00112111001100101111111101111111111011100000-00--0----00000--110000-0000-00---0-0--0---------0---*
*Aurelia 001100111111100000-00112111001100101111111101111111110110110000 0-00--0----00000--110000-0000-00---0-0--0---------0---*
*Platynereis 00110011111110000 0-00113110101100101111312211011000000----0000010110101-10000021012111001110-00000000-0000000000-0---*
*Pinctada 00110011111110000 0-00113110101100101111312211011000000----00000101101011-00000210121111110000-00000000-0000000000-0---*
*Tribolium 00110011111110000 0-00113110101100101111312211011000000----00000101101111-00000210120000010011111100 00-0000000000-0---*
*Epiperipatus 00110011111110000 0-00113110101100101111312211011000000----00000101101111-00000210120000010011110011 00-0000000000-0---*
*Priapulus 00110011111110000 0-00113110101100101111312211011000000----0000010110101-10111121012000001001111000000-0000000000-0---*
*Saccoglossus 00110011111110000 0-00113110101100101111312211011000000----00000111100000010000221130000000000-00000000-001110000000000*
*Strongylocentrotus 00110011111110000 0-00113110101100101111312211011000000----00000111100000010000221130000000000-00000001111000000000000*
*Branchiostoma 00110011111110000 0-00113110101100101111313211011000000----00000111110000010000221130000000000-00000010-000001110100000*
*Ciona 00110011111110000 0-00113110101100101111312111011000000----00000111110000010000221130000000000-00000000-000001111100000*
*Ambystoma 00110011111110000 0-00113110101100101111313211011000000----0000011111?000010000221130000000000-00000010-000001110111111*

# Appendix 3:

## Publications arising from this thesis. Articles first published in:

**Dunn, F. S.** and Liu, A. G. Fossil Focus: The Ediacaran Biota. *Palaeontology Online.* ***7***(1), 1–15.

Hoekzema, R.S., Brasier, M.D., **Dunn, F.S.** and Liu, A.G., 2017. Quantitative study of developmental biology confirms Dickinsonia as a metazoan. *Proceedings of the Royal Society B: Biological Sciences*, ***284***(1862), 20171348.

**Dunn, F. S.,** Liu, A. G. and Donoghue, P. C. J. 2018. Ediacaran Developmental Biology. *Biological Reviews.* ***93***(2), 914–932.

**Dunn, F.S.,** Wilby, P.R., Kenchington, C.G., Grazhdankin, D.V., Donoghue, P.C. J. and Liu, A.G., 2018. Anatomy of the Ediacaran rangeomorph *Charnia masoni*. *Papers in Palaeontology*. **5**(1), 157–176.

Kenchington, C.G., **Dunn, F.S**. and Wilby, P.R., 2018. Modularity and overcompensatory growth in Ediacaran rangeomorphs demonstrate early adaptations for coping with environmental pressures. *Current Biology*, ***28***(20), 3330-3336.

**Dunn, F. S.** and Liu, A. G. 2019. Viewing the Ediacaran Macrobiota as a failed experiment is unhelpful. *Nature Ecology and Evolution.*

Wood, R., Liu, A.G., Bowyer, F., Wilby, P.R., **Dunn, F.S**., Kenchington, C.G., Cuthill, J.F.H., Mitchell, E.G. and Penny, A., 2019. Integrated records of environmental change and evolution challenge the Cambrian Explosion. *Nature Ecology & Evolution*.

**Dunn, F. S.,** Liu, A. G. and Gehling, J. G. Anatomical and ontogenetic reassessment of the Ediacaran frond *Arborea arborea* and its placement within total group Eumetazoa. *Palaeontology.* **Dunn, F.S.,** Wilby, P.R., Kenchington, C.G., Grazhdankin, D.V., Donoghue, P.C. J. and Liu, A.G., 2018. Anatomy of the Ediacaran rangeomorph *Charnia masoni*. *Papers in Palaeontology*. **5**(1), 157–176.

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# Fossil Focus: The Ediacaran Biota

by Frances S. Dunn[*1] and Alex G. Liu[2]

## Introduction:

The Ediacaran period, from 635 million to 541 million years ago, was a time of immense geological and evolutionary change. It witnessed the transition out of an ice-house climate, the break–up of one supercontinent (Rodinia) and the assembly of another (Gondwana), a major meteorite impact (the Acraman event) and unprecedented shifts in global ocean chemistry that included a significant rise in oxygen concentrations (Fig. 1A). Rocks from the Ediacaran also record the appearance of a diverse (species-rich) group of large, morphologically complex lifeforms: the Ediacaran biota. These organisms were globally abundant from about 571 million to 541 million years ago. To our modern eyes, many Ediacaran fossils look strange and unfamiliar, and they have puzzled palaeontologists for decades. Determining the position of these organisms in the tree of life is one of the biggest unresolved challenges in palaeobiology.

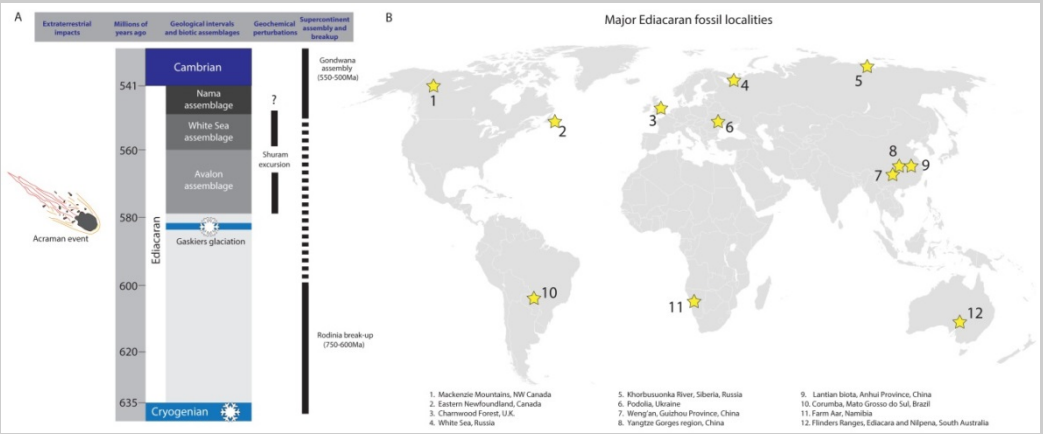


Figure 1 — Major events during the Ediacaran period, and the main palaeontological Ediacaran field localities. A, Key tectonic, geochemical and extraterrestrial events during the Ediacaran period (635 million to 541 million years ago), and the approximate ranges of the Avalon, White Sea and Nama biotic assemblages of soft-bodied Ediacaran macrofossils. The Shuram excursion is the largest recorded change in the carbon-isotope record in Earth history, and indicates a substantial shift in the global carbon cycle. However, the precise timing of this event is unclear. B, Notable Ediacaran fossil localities. All are macrofossil sites, except Weng'an, which houses the well-known Doushantuo microfossil assemblages. Credit: F. Dunn and A. Liu.

For many years, evidence from the fossil record seemed to indicate that animals appeared suddenly (geologically speaking) in an event termed the Cambrian explosion. Small shelly fossils, exotic soft-bodied invertebrates, trilobites and abundant burrows all have their first appearances in layers of rock from the Cambrian period, suggesting that animal life evolved and rapidly diversified between 540 million and 520 million years ago. However, although events in the early Cambrian are undoubtedly

relevant to our understanding of the diversification of the animal groups, major discoveries relating to their origins are being made further back in time, in the Ediacaran period. The Ediacaran contains evidence for a number of important evolutionary milestones, including fossils thought to represent evidence of the first animal movement, biomineralization (the formation of hard shells or spicules), predation and reefs. Perhaps the most infamous Ediacaran fossils, however, are those of the Ediacaran biota.

Fossils of the Ediacaran biota preserve a record of large (up to 2 metres), biologically complex, mostly soft-bodied organisms, and are most commonly found as impressions of their external surfaces. The study of Ediacaran fossils has had a relatively brief history. It was only in the 1950s that they were confirmed to be older than the Cambrian, and the Ediacaran System to which they are now assigned was formally defined only in 2004. Importantly, their often unusual body plans mean that even very basic questions, such as 'what were the Ediacaran biota?', are still controversial. We need to answer such questions if we are to understand the early evolution of animals and, more broadly, the diversification and development of multicellular life. This article will briefly describe what we mean by the Ediacaran biota; look at previous suggestions of what they are likely to have been; and summarize the most recent thinking on how their appearance and (apparent) disappearance in the fossil record may relate to geological events.

## Introducing the Ediacaran macrobiota:

Between the 1840s and 1870s, interesting impressions were found in England and Newfoundland, Canada, on bedding planes that pre-dated known Cambrian fossils (Fig. 2B–C). It was not clear to their discoverers whether these often circular structures were truly the remains of living organisms, or were the result of unrelated sedimentary processes. The widely held opinion at the time was that there was no life before the Cambrian; older rocks were called Azoic, meaning 'without life', and the known fossil record strongly suggested that life exploded from nowhere into an astounding diversity of forms during the Early Cambrian, around 520 million years ago. This paradigm discouraged the early discoverers from seriously considering that their impressions could have been biological in origin, but contradicted the long ancestry of complex life predicted by Darwin's theory of evolution by natural selection. In the 1930s and 1940s, a range of more complex impressions were found in Russia, Namibia and South Australia. Although it was clear that these fossils recorded biological remains, their actual age could not be determined. The Russian material lay in sediments that had previously been mapped as dating from the Devonian period (419 million to 359 million years ago), whereas in Australia, the possibility that the fossils belonged to the lowest Cambrian could not be ruled out.

The situation changed in 1957 with the discovery and publication of a fern-like fossil named *Charnia masoni* (Fig. 2A), which was found on a bedding plane in England that was demonstrably older than the Cambrian. The similarities between *Charnia* and some Australian and Russian fossils (particularly other fern-like forms) enabled palaeontologists to recognize that globally distributed communities of soft-bodied organisms had existed, and thrived, well before the famous Cambrian explosion, vindicating Darwin's predictions. The organisms were collectively termed the Ediacara biota, after the Ediacara Hills in South Australia, from which some of the specimens had been discovered.

Since those early discoveries, members of the Ediacara biota have been found all over the world (Fig. 1B). They have been joined by a variety of other late Ediacaran fossils that are not found at the original Ediacara site, and do not represent the remains of originally soft-bodied organisms.


Figure 2 — Fossils discovered between 1840 and 1957 in rocks of 'Azoic' age. A, *Charnia masoni*, discovered by schoolchildren in Charnwood Forest, Leicestershire, UK, in the 1950s. This specimen is the holotype (type specimen) of *Charnia masoni*, and is housed at the New Walk Museum, Leicester. B, *Aspidella terranovica*, from St John's in Newfoundland, Canada. C, 'Ring fossils' from the 'ring pit', Charnwood Forest, first documented in the 1840s. These disc-shaped fossils are now recognized to be the anchoring holdfasts of frond-like organisms. Scale bars, 10 mm (A–B) and 50 mm (C). Credit: F. Dunn and A. Liu.

As a result, 'Ediacara biota' has become a less clear-cut term, and in this article we use 'Ediacaran macrobiota' to refer to all large fossils of late Ediacaran age, soft-bodied or otherwise. Further confusion has arisen because different research groups have previously used different systems to categorize rocks of Ediacaran age (for example, the Sinian System in China and the Russian Vendian System). These systems did not precisely correlate with one another, but the decision in 2004 to use the Ediacaran System as the internationally agreed system has mostly resolved this issue.

Before concentrating on Ediacaran macrofossils, we emphasize that life had already achieved considerable diversity well before their appearance. In addition to a Precambrian record of microbes

(including stromatolites, thrombolites, and microbial mats) spanning around 3 billion years, fossils from the Cryogenian period (720 million to 635 million years ago) and Tonian period (~1 billion to 720 million years ago) suggest that groups including foraminifera, amoebae and red algae were present from around 700 million to 800 million years ago. There are eukaryotic embryos from the Doushantuo Formation of China some 600 million years ago (which some researchers claim to be animal), alongside microfossils of acritarchs and algae. In short, the Ediacaran macro-organisms would have shared the oceans with a host of other lifeforms, and represent only one component of a diverse and complex biosphere.

The oldest Ediacaran macrofossils may belong to the Lantian biota of China. Here, groups of large algae a few centimetres long are joined by conical organisms with 'tentacle-like' structures (such as *Lantianella*, Fig. 3), which have been compared to cnidarian polyps. The Lantian fossils could be as old as 600 million years, but their age is yet to be precisely pinpointed, and they might have been younger. As a result, fossils of what is known as the Avalonian biota, from the United Kingdom and Newfoundland, are often said to document the oldest communities of diverse macroscopic (big enough to be seen without a microscope) complex organisms, appearing in the fossil record around 571 million years ago. The vast majority of the Avalonian organisms were soft-bodied, frond-like in shape, and lived stationary lives anchored to the sea floor (Fig. 4). They lived in darkness in deep-marine environments, either reclining on the sediment or elevated into the water column. The frond-like forms, including *Charnia* and *Fractofusus* (Figs 2A, 4D), dominate Ediacaran fossil assemblages spanning almost 20 million years, with only a few non-frond-like groups for company (including the triangular form *Thectardis*, Fig. 4A). No Ediacaran fronds have yet been convincingly identified as animals, but rare trace fossils (Fig. 5A) and impressions of non-frond-like organisms (for example, *Haootia*, Fig. 4C) may hint at the presence of early animals.


Figure 3 — Macrofossils from the Lantian biota, Anhui Province, China. Specimens are housed in the collections of the Nanjing Institute of Geology and Palaeontology. A, *Lantianella laevis* (at left; NIGP163377), and a larger conical form. B, *Lantianella annularis*, NIGP163384. Scale bars, 10 mm. Credit: F. Dunn and A. Liu.

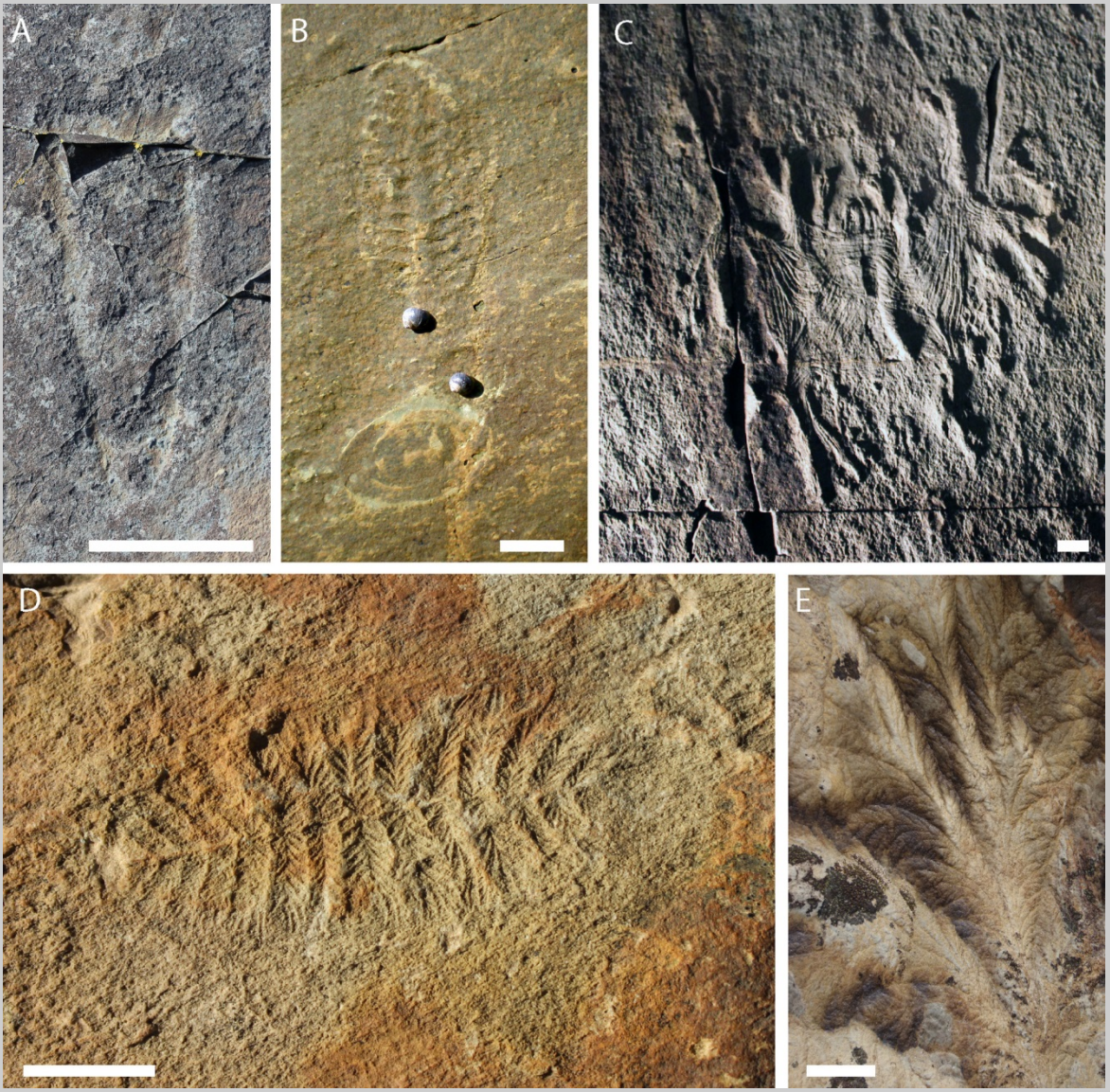


Figure 4 — Avalonian taxa from Newfoundland, Canada. A, *Thectardis*, Drook Formation. B, *Charniodiscus*, a frondose fossil from the Mistaken Point Formation. C, *Haootia*, a possible muscle-bearing cnidarian from the Fermeuse Formation. This holotype specimen is housed at The Rooms Provincial Museum, St. John's. D, *Fractofusus*, a frondose fossil from the Briscal Formation, Mistaken Point Ecological Reserve World Heritage Site. E, Partial *Bradgatia*, a rangeomorph from the Trepassey Formation of the Bonavista Peninsula. All specimens except C remain in the field. Scale bars, 50 mm (A) and 10 mm (B–E). Credit: F. Dunn and A. Liu.

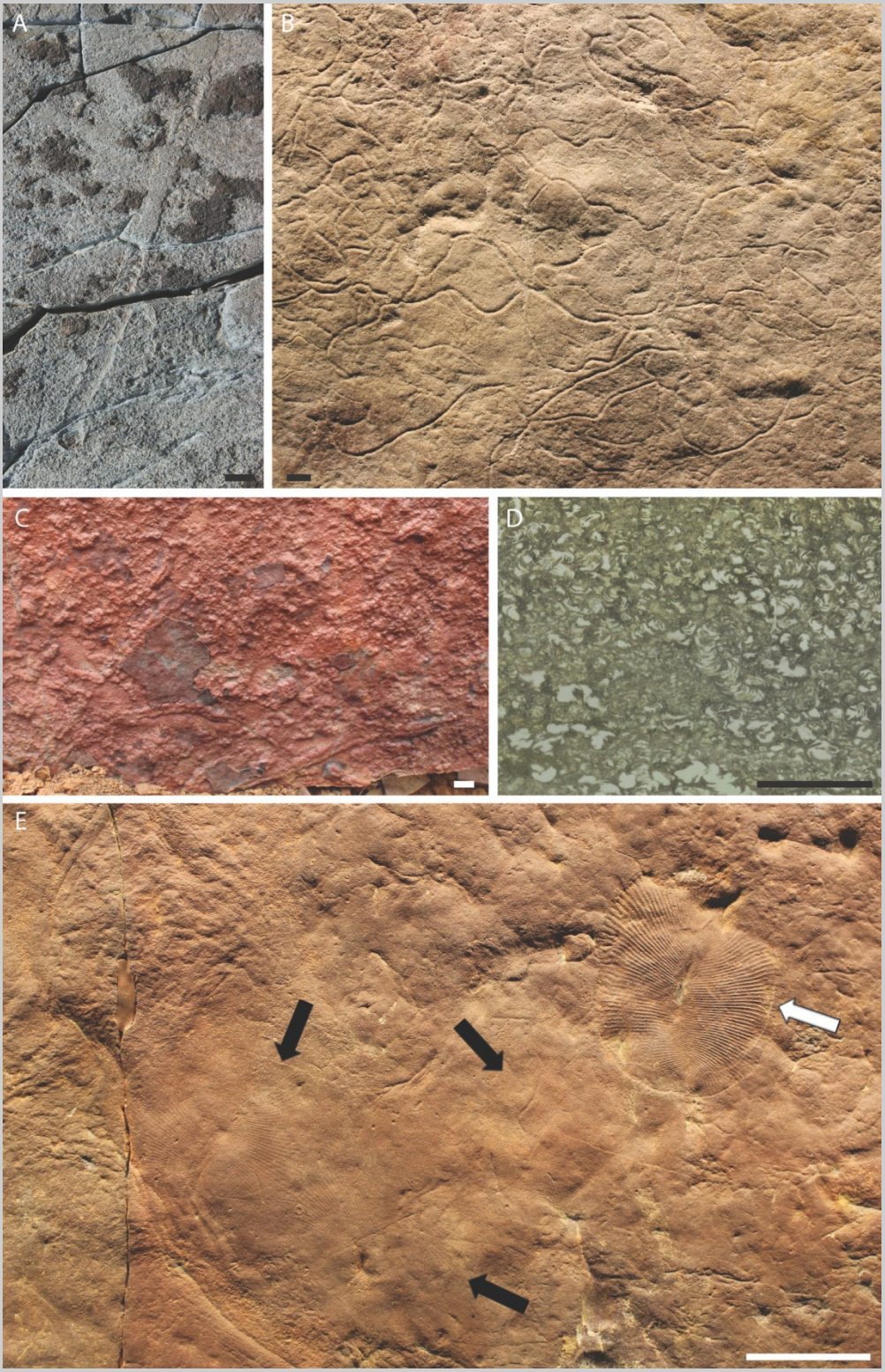


Figure 5 — Trace fossils from the upper Ediacaran period. A, Surface horizontal trace, Mistaken Point Formation (about 565 million years old), Newfoundland. B, *Helminthoidichnites* trace fossils made beneath a microbial mat, Ediacara Member, South Australia (around 560 million years old), South Australia Museum specimen SAM P42142. C, Bi-lobed traces considered to have been made by bilaterian animals, Shibantan Member, South China (about 555 million years old). D, Potential bioturbation in the Khatyspyt Formation, Arctic Siberia (about 553 million years old). E, *Epibaion* impressions (black arrows) associated with *Dickinsonia costata* (white arrow, SAM P49377) and interpreted as evidence for active movement by *Dickinsonia*. Ediacara Member, South Australia. Scale bars, 10mm (A–D) and 50 mm (E). Credit: F. Dunn and A. Liu.

Around 560 million years ago, we see a sharp increase in the apparent diversity of Ediacaran macrofossils worldwide. The fronds become less diverse, but are joined by a variety of new forms, including teardrop-shaped organisms such as *Parvancorina* (Fig. 6B), 'segmented' forms such as *Spriggina* (Fig. 6C) and circular forms such as the tri-radial *Tribrachidium* (Fig. 7B). These organisms lived in shallow seas, and today are found most abundantly in South Australia and the White Sea of Russia. They include a number of groups that have previously been interpreted (with varying degrees of confidence) as early animals, such as *Arkarua* (echinoderms), *Eoandromeda* (ctenophores) and the tongue-twisting *Palaeophragmodictya* (compared to sponges; Fig. 7C). Importantly, some of these organisms, like *Dickinsonia* (Figs 6A, 8A) and *Kimberella* (Figs 6D, 8C), are often found close to trace fossils possibly created by movement and feeding (Fig. 5E), which are two key animal characteristics. Other trace fossils of a similar age document the movement of small organisms beneath microbial mats (Fig. 5B), and potentially (from Siberia) even vertical burrowing and sediment mixing (Fig. 5D). Macroalgae (such as *Flabellophyton*; Fig. 9A) are also present in these shallow marine settings, together with a variety of large tubular organisms, often composed of ring-like segments, some of which might have reproduced sexually (for example *Funisia*; Fig. 7A).

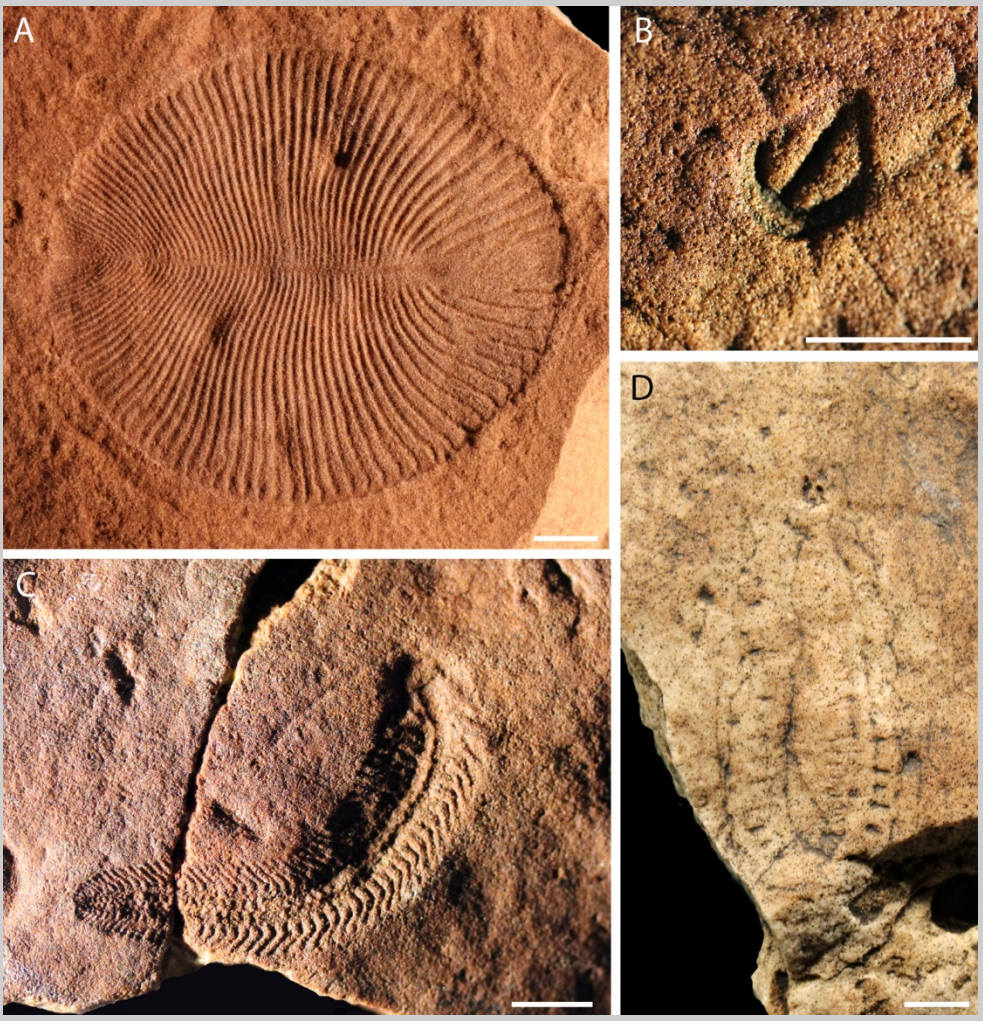


Figure 6 — Ediacaran fossils from the Ediacara Member, South Australia. All specimens reside at the South Australia Museum. A, *Dickinsonia*, SAM P40135. B, *Parvancorina*, SAM P40695. C, Two specimens of *Spriggina*, SAM P29802 and P29803. D, *Kimberella*, SAM P48935. Scale bars, 10 mm. Credit: F. Dunn and A. Liu.


Figure 7 — Further soft-bodied Ediacaran organisms from the Ediacara Member, South Australia. All specimens reside at the South Australia Museum. A, *Funisia*, SAM P40726. B, *Tribrachidium*, SAM P12898 (holotype). C, *Palaeophragmodictya*, SAM P48140. D, *Eoandromeda*, SAM P44349. E, Arkarua, SAM P49266. F, *Palaeopascichnus*, SAM P36854d. G, *Nemiana*, SAM P49342. Scale bars, 10 mm. Credit: F. Dunn and A. Liu.

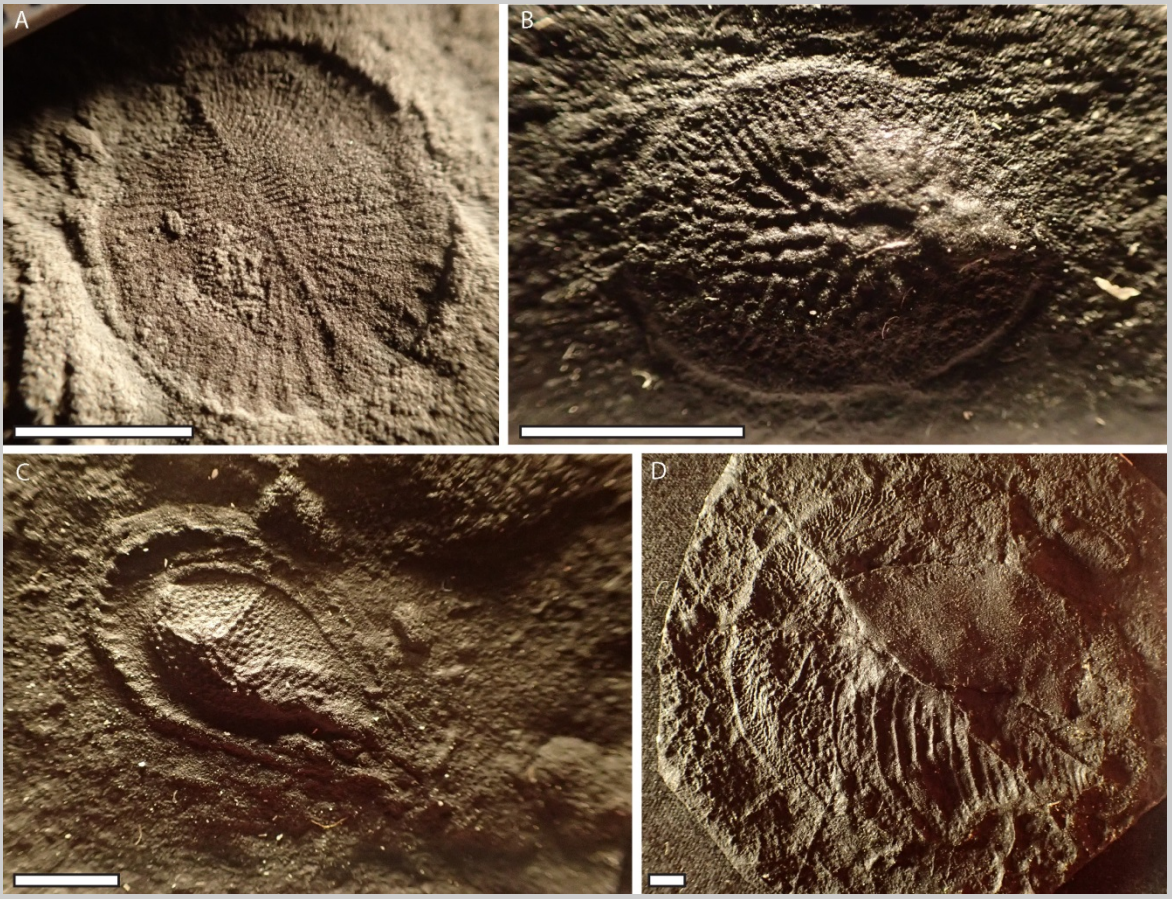


Figure 8 — Casts of soft-bodied Ediacaran organisms from the White Sea of Russia. All photographed specimens reside at the Royal Ontario Museum. A, *Dickinsonia*, ROM 54231. B, *Solza*, ROM 62397. C, *Kimberella*, ROM 62392. D, *Yorgia*, ROM 62387. Scale bars, 5 mm. Credit: F. Dunn and A. Liu.


Figure 9 — Macroalgal taxa in the late Ediacaran period. A, *Flabellophyton*, Ediacara Member, South Australia, SAM P35701. B, *Liulingjitaenia*, South Australia, SAM P48771. C, *Longifuniculum*, field specimen, Miaohe Member, South China. D, *Konglingiphyton*, Miaohe Member, South China. Scale bars, 10 mm (A–B) and 5 mm (C–D). Credit: F. Dunn and A. Liu.

The White Sea/Australian biota persisted until about 550 million years ago, and represent the pinnacle of Ediacaran macroscopic diversity. After this, the number of soft-bodied groups declines in Ediacaran assemblages worldwide. Bag-shaped organisms that seem to have lived within the sediment of the sea floor, such as *Pteridinium* and *Ernietta*, become the most common soft-bodied groups, but these bizarre organisms had very unusual body plans, being composed of aligned tubes that may have been filled with sand during life (Fig. 10). Tubular fossils remained common (such as *Wutubus* and *Corumbella*; Fig. 11). Perhaps the most striking new arrivals were biomineralizing organisms with calcium carbonate skeletons, such as *Cloudina* (Fig. 11B) and *Namacalathus*. These organisms constructed the earliest reefs. Some *Cloudina* have also been found with holes in their shells, possibly indicating that they were being preyed upon.

Taken together, the Ediacaran macrofossil record suggests that by the start of the Cambrian, many important ecological and physiological innovations that would shape ecosystems in the Phanerozoic eon (from 541 million years ago to the present), such as predation, movement, reef-building and perhaps even bioturbation, had already evolved. Although we are hampered by the limitations of the fossil record, and by insufficient understanding of the age of many Ediacaran fossil localities, researchers are making progress in tackling questions about the patterns and processes that governed the distribution of Ediacaran organisms in time and space. However, a more fundamental question remains: what sort of organisms were the Ediacaran macrobiota?


Figure 10 — Ediacaran macrofossils from the Nama Group, Namibia, around 545 million years old. A, *Pteridinium*. B, *Ernietta*. C, *Swartpuntia*. Scale bar in C, 10 mm. Credit: M. Laflamme (A–B) and J. Hoyal Cuthill (C).

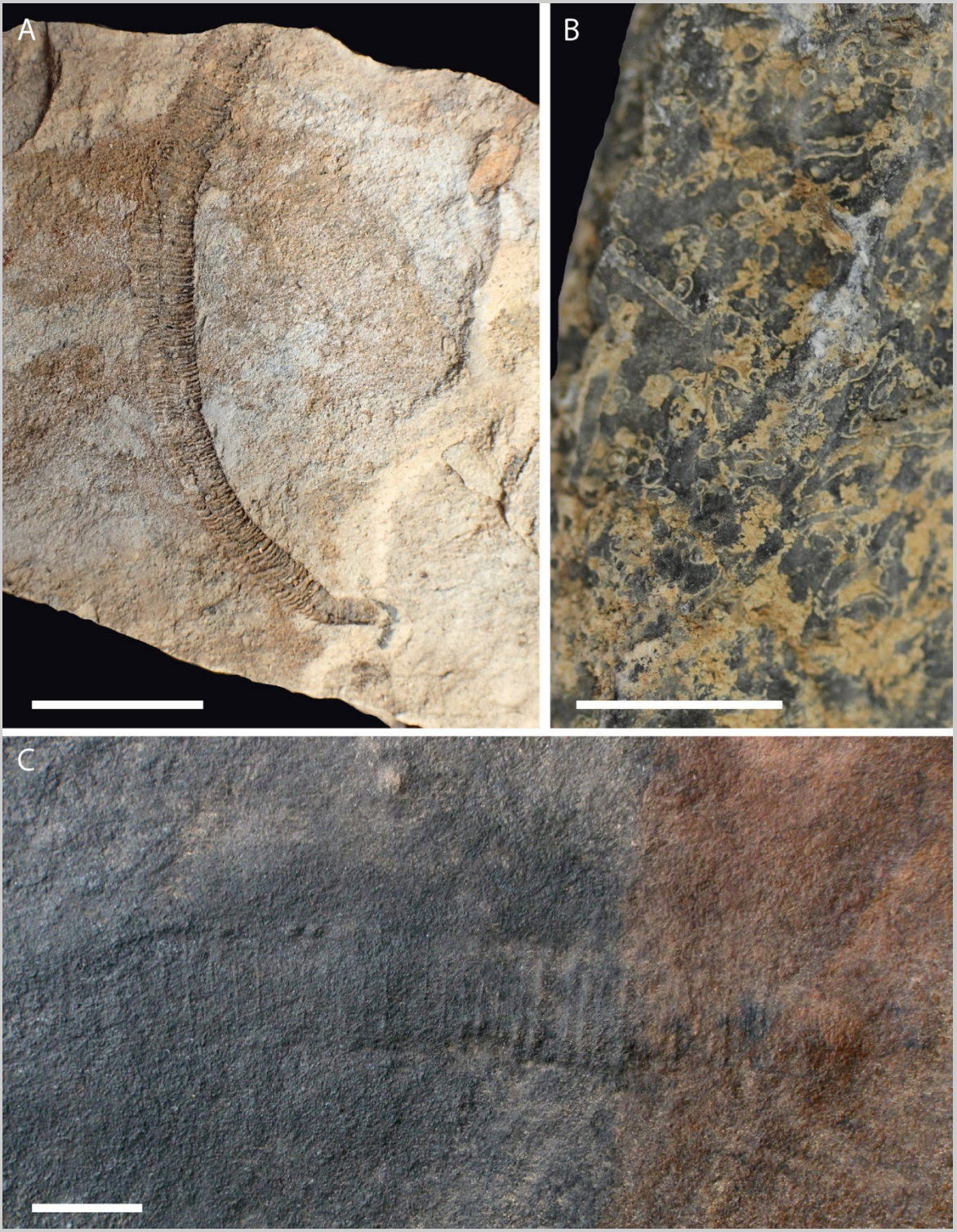


Figure 11 — Tubular taxa of the latest Ediacaran period. A, The possible cnidarian *Corumbella*, field specimen from the Tamengo Formation, Brazil. Corumbella has a seemingly flexible organic exoskeleton. B, *Cloudina*, from the Nama Group, Namibia, with a calcium carbonate cone-in-cone construction. C, *Wutubus*, field specimen from the Shibantan Member, South China. *Wutubus* was a seemingly soft-bodied, non-mineralized organism. Scale bars, 10 mm. Credit: F. Dunn and A. Liu.

## Resolving the biological affinities of the Ediacaran macrobiota:

When first described in the 1960s and 1970s, members of the original Ediacara biota were largely considered to be ancient animals. Many frond-like groups were compared to sea-pens (a form of soft coral). *Dickinsonia* and *Spriggina* were thought to be annelid worms, and a variety of circular impressions (now recognized to be anchoring structures that attached Ediacaran fronds to the sediment) were interpreted as jellyfish. Those views culminated in Martin Glaessner's 1985 book *The Dawn of Animal Life*. However, palaeontologists such as Hans Pflug, Mikhail Fedonkin and Dolf Seilacher had already begun to question whether Ediacaran fossils really recorded the impressions of creatures belonging to extant animal groups in the 1970s and 1980s. They suggested that many members of the soft-bodied Ediacaran macrobiota may have been more closely related to each other than to any groups of organisms alive today. Seilacher took this argument furthest, reconstructing organisms such as *Charnia*, *Dickinsonia* and *Tribrachidium* as being made up of modular, self-repeating units, and placing them in their own extinct clade, the Vendozoa. This idea stimulated interest in the fossils, and led to a wide range of other suggestions, including that the organisms might have been fungi, protists, bacterial colonies or (most dubiously) lichens, rather than animals or vendozoans. As a result, to a non-specialist the discussion of Ediacaran fossils can seem extremely confusing and discordant, but since the turn of the millennium there has been a growing acceptance that the soft-bodied Ediacaran macrobiota should not be treated as a single monophyletic entity, all descended from one common ancestor. Instead, the biota reflect a diverse group of organisms that include crown- and stem-group members of several kingdoms and phyla (see Box 1), including early animals.

New technologies and approaches to studying these organisms are rapidly improving our knowledge of individual groups. These techniques include tomographic scanning, topographic analysis, and laser scanning for better visualization of morphological features (see Video 1), as well as modern ecological and geochemical methods.

Perhaps the fossils most likely to record Ediacaran animals are the mollusc-like organism *Kimberella* (Figs 6D, 8C), and *Dickinsonia* (although its precise position in the animal tree is unclear, with suggestions that it could be a placozoan, ctenophore, cnidarian or a bilaterian all proposed; Figs 6A, 8A). Several tubular fossils, such as *Corumbella*, have been compared to cnidarians, and some of the oldest candidate animal fossils include: the possible staurozoan *Haootia* at around 560 million years ago; the potentially older fossils *Lantianella* and *Xiuningella* from the Lantian biota; and the considerably more ancient putative sponge *Eocyathispongia* at 600 million years ago. Trace fossils likely to have been formed by animals (Fig. 5), probable sponge biomarkers, and predictions from modern DNA analysis (molecular clocks) all support the idea that animals existed before the Cambrian, and it should therefore not be surprising if many of the Ediacaran macrobiota do turn out to be animals. However, considerable work remains to confirm the validity of animal and other interpretations, and as palaeontologists we need to provide positive and robust evidence in order to determine the biological affinities of these fossils.

## Box 1: Crown group or stem group?

Evolutionary relationships between organisms, both living and extinct, are studied as part of a discipline called phylogenetics. Phylogenetics attempts to identify related biological groups and construct a tree of life, using the assumption that closely related organisms should be more similar to each other than to more distantly related groups, or taxa. Organisms with common features, or characters, are assigned to groups called clades. For example, wolves and donkeys are both covered in fur and have mammary glands (characters that are shared by all mammals), and so are more closely related to each other than either is to, for example, a snail. But follow the tree back, and wolves, donkeys and snails will eventually share common characters, and can be assigned to the same clade (in this case, the Animalia).

We use specific terminology to describe the relationship between a fossil taxon and living organisms:

- The 'crown group' is made up of the last common ancestor of all extant (living) members of a group, and all of its descendants (many of which will be extinct).
- The 'stem group' consists of extinct lineages that fall outside the crown group, but are considered to be more closely related to that group than to any others.
- If we combine both of these groups, we have the 'total group': all the living members of the group and all fossil organisms considered to be more closely related to that group than to any other.

These terms help palaeontologists to relate long-dead organisms to extant groups. Where we place a fossil in a group depends on the combination of characters it shows. To lie in the crown group, an organism typically must possess all characters found in that group, or have been known to have once had them, and then lost them ('lost them secondarily'). Where an organism has some but not all of the characters of a group, it might be more likely to belong in the stem. The term total group is particularly useful if we know that a fossil belongs to a particular clade, but we don't know the relationships between extant groups well enough to be able to say whether the fossil belongs in the crown or stem group (Fig. 12).

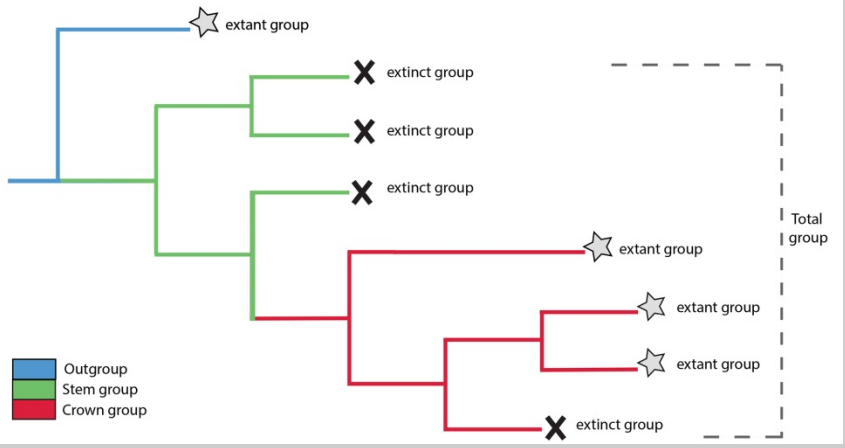


Figure 12 — An illustration of the concepts of total, stem and crown groups. An outgroup is an organism to which the group of interest is closely related to. Credit: F. Dunn and A. Liu.

Determining where the Ediacaran macro-organisms lie in the tree of life is difficult, because they have few preserved characters that could help us to assign them to any particular clade. Recent studies have suggested that many Ediacaran macrofossils may have been stem-group members of various animal phyla, or even stem-group animals.

## Why did the Ediacaran macrobiota appear in the latest Precambrian?

Even if we can positively identify the Ediacaran macrobiota, questions remain about how the organisms lived, their relationships to one another, their interactions within their ecosystems and their impact on the wider biosphere. One broader question with implications for the wider Earth system is why did they appear in the fossil record at around 571 million years ago? The rock record reveals a short-lived glacial episode, the Gaskiers glaciation, only a few million years before the earliest Ediacaran macrobiota show up in the fossil record (Fig. 1A), but this may not have been a global event. Researchers have speculated that a release from ice-house conditions would have melted icecaps and released vast quantities of nutrients into the oceans, supporting blooms of microbes called cyanobacteria and triggering an increase in atmospheric oxygen levels. Oxygenation of the oceans may have let animals thrive and diversify, but several researchers dispute this. Some doubt that the timing of the oxygen rise correlates with the appearance of the earliest animals, and others have suggested that the presence of animals may itself have oxygenated the planet. Current research suggests that stability in oxygen levels may have been more important in creating suitable conditions for the evolution of complex life than how much oxygen there actually was. An alternative, biological, explanation for the appearance of the Ediacaran macrobiota is an explosion in genetic diversity, not necessarily because the genes themselves diversified, but because animals evolved new genetic 'machinery' that controlled how genes acted. Unravelling these factors is difficult, but will be essential if we are to determine what led the Ediacaran macrobiota to appear when they did.

Equally notable is the apparent disappearance of the Ediacaran macrobiota from the rock record at the start of the Cambrian, with only a handful of Ediacaran 'survivors' having been described from Cambrian rocks. Possible explanations for this disappearance have included: an extinction event caused by the appearance and diversification of the first predators; the removal of unique conditions that favoured the fossilization of soft-bodied organisms; and out-competition by better-adapted organisms. The latter scenario has been heavily influenced by the idea of ecosystem engineering — the alteration of an environment through the activities of organisms, such as burrowing or recycling nutrients — which may have resulted in the removal of microbial mats (a source of food and sediment stability for some of the Ediacaran macro-organisms). This ecosystem-engineering model has recently been supported by large scale analyses of multiple Ediacaran localities and their fossils.

## Summary:

Ediacaran fossils undoubtedly record important steps in eukaryotic evolution, and together with the Cambrian fossil record they reveal an interval of biological innovation and diversification on a scale unparalleled in Earth history. Although the precise identities of many Ediacaran forms remain elusive, our understanding of both individual organisms and wider ecosystems is improving at a remarkable rate. New and exciting fossils are being discovered every year, and many more are yet to be formally described and studied. Continued expansion of research to consider the full range of Ediacaran organisms (rather than only a handful of iconic groups), and to use new techniques and data sets from other geological and biological disciplines, offers our best hope of understanding the true place of these remarkable fossils in the evolutionary history of our planet.

## Suggestions for further reading:

Budd, G. E. & Jensen S. A critical reappraisal of the fossil record of the bilaterian phyla. *Biological Reviews* **75**, 253–295 (2000). DOI: 10.1111/j.1469-185X.1999.tb00046.x

Erwin, D. H. *et al*. The Cambrian conundrum: early divergence and later ecological success in the early history of animals. *Science* **334**, 1091–1097 (2011). DOI: 10.1126/science.1206375

Fedonkin, M. A. *The Rise of Animals: Evolution and Diversification of the Kingdom Animalia*. (Johns Hopkins University Press, 2007).

Glaessner, M. F. *The Dawn of Animal Life: A Biohistorical Study*. (Cambridge University Press, 1985).

Laflamme, M., Darroch, S. A. F., Tweedt, S. M., Peterson, K. J. & Erwin D. H. The end of the Ediacara biota: Extinction, biotic replacement, or Cheshire Cat? *Gondwana Research* **23**, 558–573 (2013). DOI: 10.1016/j.gr.2012.11.004

Liu, A. G. Framboidal pyrite shroud confirms the 'death mask' model for moldic preservation of Ediacaran soft-bodied organisms. *PALAIOS* **31**, 259–274 (2016). DOI: 10.2110/palo.2015.095

Narbonne, G. M., Xiao, S. & Shields, G. The Ediacaran Period. In: *The Geologic Timescale*. (eds Gradstein, F. M., Ogg, J. G., Schmitz, M. & Ogg, G.) 413–435 (2012).

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# Ediacaran developmental biology

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ABSTRACT

Rocks of the Ediacaran System (635–541 Ma) preserve fossil evidence of some of the earliest complex macroscopic organisms, many of which have been interpreted as animals. However, the unusual morphologies of some of these organisms have made it difficult to resolve their biological relationships to modern metazoan groups. Alternative competing phylogenetic interpretations have been proposed for Ediacaran taxa, including algae, fungi, lichens, rhizoid protists, and even an extinct higher-order group (Vendobionta). If a metazoan affinity can be demonstrated for these organisms, as advocated by many researchers, they could prove informative in debates concerning the evolution of the metazoan body axis, the making and breaking of axial symmetries, and the appearance of a metameric body plan. Attempts to decipher members of the enigmatic Ediacaran macrobiota have largely involved study of morphology: comparative analysis of their developmental phases has received little attention. Here we present what is known of ontogeny across the three iconic Ediacaran taxa *Charnia masoni*, *Dickinsonia costata* and *Pteridinium simplex*, together with new ontogenetic data and insights. We use these data and interpretations to re-evaluate the phylogenetic position of the broader Ediacaran morphogroups to which these taxa are considered to belong (rangeomorphs, dickinsoniomorphs and erniettomorphs). We conclude, based on the available evidence, that the affinities of the rangeomorphs and the dickinsoniomorphs lie within Metazoa.

*Key words*: Metazoa, development, evolution, Ediacaran, Bilateria, Eumetazoa.

CONTENTS



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## I. INTRODUCTION

Among multicellular eukaryotes, Metazoa are unique in exploring a broad range of diverse body plans. Assisted by their ability to undergo coordinated embryogenesis (Valentine, Tiffney, & Sepkoski, 1991), and free from the restrictions of rigid cell walls, animals have evolved well over 100 distinct cell types [compared to ∼7 in fungi and kelps and ∼30 in higher plants (Bonner, 1988)], and have arranged them into diverse tissue types, physiological systems, and morphological structures. Animals are therefore among the most biologically complex organisms. Elucidating the developmental processes that underpin this complexity is a major challenge for contemporary evolutionary and developmental biology.

Molecular clock estimates suggest that animals originated ∼700–800 million years ago (Ma) (dos Reis *et al.*, 2015), but unequivocal fossil evidence for animals is not found until closer to ∼541 Ma (e.g. Erwin *et al.*, 2011; Cunningham *et al.*, 2017). Some of the most likely candidates for early animal fossils are found within the Ediacaran Biota; an enigmatic assemblage of largely soft-bodied macroscopic organisms that spans the ∼40 million year interval immediately prior to the Cambrian Period (Fedonkin *et al.*, 2007; Cunningham *et al.*, 2017). Many of these organisms, which are typically preserved only as impressions of their external surfaces, are united by a body plan composed of self-repeating morphological units. Their fossil remains possess few morphological characters that are diagnostic of any particular phylogenetic affinity, and multiple competing hypotheses for where they lie within Eukarya have been proposed since their initial description (summarised in Xiao & Laflamme, 2009; Budd & Jensen, 2017), including the suggestion that they represent an entirely distinct Kingdom Vendobionta (Seilacher, 1989, 1992). This latter hypothesis later softened to consider the Ediacaran Biota as an extinct phylum within total-group Metazoa or total-group Eumetazoa (Buss & Seilacher, 1994); a view not substantially different from the current broad consensus that these Ediacaran organisms are allied to early-branching lineages of Metazoa or Eumetazoa (e.g. Xiao & Laflamme, 2009; Budd & Jensen, 2017). Surprisingly, in many cases this consensus does not rest on material evidence of metazoan affinity but, rather, on an implicit assumption that the organisms are total-group metazoans. As a result, Ediacaran taxa are invoked in debate on the origin and evolution of metazoan developmental novelties, including the specification of primary body axes, the making and breaking of axial symmetries, and the appearance of metamerism and/or segmentation (e.g. Malakhov, 2004). Determining the correct phylogenetic position of Ediacaran macrofossil taxa, or even being able to provide convincing positive evidence for an unquestionably metazoan placement, is therefore a challenge with significant consequences for understanding the evolution of metazoan development and morphogenesis.

It is perhaps surprising that although developmental insights can be gleaned from Ediacaran fossil assemblages, Ediacaran developmental biology remains in its infancy. The little work that has been done, based on the premise that ontogenetic characters are considered to have been conserved across evolutionary time, demonstrates the potential power of morphogenesis in testing established hypotheses of affinity (e.g. Antcliffe & Brasier, 2007: Gold *et al.*, 2015). Investigation of morphogenesis in Ediacaran taxa also has the potential to constrain hypotheses of developmental evolution associated with the evolutionary emergence of animals, and to test models of trait evolution that are currently based only on theoretical predictions. Here we review the existing data and interpretations regarding morphogenesis in key Ediacaran macro-organisms, and use this information to constrain hypotheses of their evolutionary relationships to extant eukaryotic groups.

## II. THE SEMANTICS OF EDIACARAN MORPHOGENESIS

Describing ontogeny in fossil organisms can be problematic (e.g. Hone, Farke, & Wedel, 2016). Many extant organisms display some form of ontogenetic shift (Paris & Laudet, 2008) and this is often used to distinguish between juvenile and adult individuals. However, such shifts are difficult to identify with certainty in extinct organisms, and have typically not been recognised in Ediacaran fossil taxa, whose adult and juvenile stages have largely been distinguished based only on the size of the specimens (e.g. Liu *et al.*, 2012). Moreover, many extant clades, including several metazoan groups to which members of the Ediacaran macrobiota have been compared, exhibit a morphologically distinct juvenile stage that bears little resemblance to the adult form (e.g. the planula larvae of Cnidaria). Discrimination of adults and juveniles among Ediacaran macrofossils is not, therefore, something that we can necessarily expect to achieve, and such terms should be avoided. The alternative use of 'size classes' is both arbitrary and potentially subject to change as new specimens are described. Allocation of specimens to 'generations' is another possibility (see Mitchell *et al.*, 2015), but at least some bedding-plane assemblages of Ediacaran macro-organisms are considered to reflect only single generations, despite large variance in size (Darroch, Laflamme, & Clapham, 2013; although see Wilby, Kenchington, & Wilby, 2015). The simplest and most defensible strategy is to consider how the size of a fossil relates to smaller and larger specimens of the same species, and to make the reasonable assumption that larger individuals would have been older, or at least further developed, than smaller individuals (see Fedonkin, 2002; Narbonne, 2004; Flude & Narbonne, 2008).

Understanding the difference between pattern and process is also essential when considering growth in fossil taxa. It is clear that many Ediacaran taxa were composed of multiple units, which have at various points been termed branches, modules, units, isomers or segments. All of the taxa that we address have been considered to grow either by inflation (wherein a particular unit increases in size), 'insertion'

Table 1. Summary of inflationary and 'insertional' (here renamed 'differentiation', see Section II for details) styles of growth across taxa belonging to the Ediacaran morphogroups Rangeomorpha, Dickinsoniomorpha and Erniettomorpha (*sensu* Erwin *et al.*, 2011). Inflation is documented as minimal (if the organism is considered to grow almost exclusively by 'insertion'), allometric (if units of the organism appear to inflate at different rates or to different degrees), isometric (if units of the organism appear to inflate at a constant rate to one another, maintaining overall shape), or simply present (if no further information on the degree of inflation is given). Differentiation ('insertion') is either noted as observed or not-observed. Empty cells represent the absence of previously published data

| Morphotype | Taxon | Inflation | Differentiation | References |
|---|---|---|---|---|
| Rangeomorph | *Charnia masoni* | Allometric | Observed | Brasier, Antcliffe, & Liu (2012) and Antcliffe & Brasier (2008) |
| Rangeomorph | *Vinlandia antecedens* | | | |
| Rangeomorph | *Trepassia wardae* | Minimal | Observed | Narbonne *et al.* (2009) |
| Rangeomorph | *Beothukis/Culmofrons plumosa* | Present | Not-observed | Laflamme, Flude, & Narbonne (2012) and Liu, Matthews, & McIlroy (2016) |
| Rangeomorph | *Beothukis mistakensis* | Allometric | Not-observed | Laflamme *et al.* (2012) and Liu *et al.* (2016) |
| Rangeomorph | *Avalofractus abaculus* | | | |
| Rangeomorph | *Fractofusus andersoni* | Isometric | Not-observed | Darroch *et al.* (2013) and Gehling & Narbonne (2007) |
| Rangeomorph | *Fractofusus misrai* | Allometric/Isometric | Not-observed | Darroch *et al.* (2013) and Gehling & Narbonne (2007) |
| Rangeomorph | *Bradgatia linfordensis* | | | |
| Rangeomorph | *Bradgatia sp.* | Present | Not-observed | Flude & Narbonne (2008) |
| Rangeomorph | *Primocandelabrum hiemaloranurn* | | | |
| Rangeomorph | *Primocandelabrum sp.* | | | |
| Rangeomorph | *Hapsidophyllas flexibilis* | | | |
| Rangeomorph | *Frondophyllas grandis* | | | |
| Rangeomorph | *Plumeropriscum hofmanni* | | | |
| Rangeomorph | *Pectinifrons abyssalis* | Present | Observed | Bamforth, Narbonne, & Anderson (2008) |
| Dickinsoniomorph | *Andiva ivantsovi* | Isometric | | Fedonkin (2002) |
| Dickinsoniomorph | *Dickinsonia costata* | Allometric | Observed | Hoekzema *et al.* (2017), Evans, Droser, & Gehling (2017), Gold *et al.* (2015), Ivantsov (2007), Retallack (2007), Runnegar (1982) |
| Dickinsoniomorph | *Dickinsonia lissa* | Present | | Ivantsov (2007) |
| Dickinsoniomorph | *Dickinsonia rex* | Present | Observed | Ivantsov (2007) and Retallack (2007) |
| Dickinsoniomorph | *Dickinsonia tenuis* | Present | Observed | Ivantsov (2007) and Retallack (2007) |
| Dickinsoniomorph | *Windermeria aitkeni* | | | |
| Dickinsoniomorph | *Yorgia waggoneri* | | Observed | Ivantsov (2007) |
| Erniettomorph | *Ernietta plateauensis* | Present | Not observed | Ivantsov *et al.* (2016) and Dzik (1999) |
| Erniettomorph | *Nasepia altae* | | | |
| Erniettomorph | *Palaeoplatoda segmentata* | | | |
| Erniettomorph | *Phyllozoon hanseni* | | | |
| Erniettomorph | *Pteridinium simplex* | Present | Observed | Grazhdankin & Seilacher (2002) |
| Erniettomorph | *Swartpuntia germsi* | | | |
| Erniettomorph | *Valdainia plumosa* | | | |

(the sequential addition of units to an organism), or a combination of these (see Table 1 to compare the published distribution of these strategies across Ediacaran taxa). However, process terms must have a basis in ontological data (Jardine, 1969) and inferences of process should be evidenced and rationalised from assemblages of individuals representing different developmental stages. New structures and units can be added during the development of multicellular organisms in a variety of patterns, but this invariably occurs through differentiation of existing cells and tissues. Insertion of units, in the sense that it is described in Ediacaran macro-organisms, does not occur in development, except in a metaphorical sense. Unfortunately, the metaphorical concept of unit insertion is at risk of being reified as a literal process in the interpretation of these fossils. Thus, we recommend use of the term 'differentiation' in place of 'insertion'. This ensures that we do not limit comparisons to only those extant taxa that show *de novo* addition of new units. We use the term 'insertion' when summarising previous developmental studies of Ediacaran taxa in the following sections, but then revert to use of 'differentiation' from Section IV onwards.

Finally, we note that previous rangeomorph taxonomic schemes have focused on assumed polarity of growth,

considering various organisms as either unipolar, bipolar or multipolar (Brasier *et al.*, 2012). However, the assumption that growth is occurring in the positions ascribed by these terms remains untested (in an ontogenetic sense) in many rangeomorphs. We prefer here to use morphologically descriptive terminology (as opposed to morphogenetically descriptive). Previous attempts at morphological description have considered fronds to be constructed of petalodia and petaloids (Laflamme & Narbonne, 2008), but such terminology has more recently been considered inappropriate, since its correct deployment is also somewhat reliant on a complete understanding of an organism's life history (Brasier *et al.*, 2012). We therefore introduce the terms 'uniterminal', 'biterminal' and 'multiterminal' as morphological descriptors of the number of distal tips the frond possesses (not including the stem or holdfast). In practical application, previous groupings of rangeomorphs remain the same, but the new terms here are based entirely on morphological features, and avoid all assumptions regarding morphogenesis.

## III. ONTOGENY IN EDIACARAN MORPHOGROUPS

To date, ∼200 Ediacaran macrofossil taxa have been described (Fedonkin *et al.*, 2007), and multiple attempts have been made to group these within sub-groups of closely related organisms. Initially, many Ediacaran taxa were considered members of extant animal clades (e.g. Glaessner, 1984), but more recently they have instead been grouped according to morphological similarity (Erwin *et al.*, 2011; Grazhdankin, 2014), with such groupings representing grades (rather than clades) of organism. We focus our study on fossils considered to belong to three widely recognised morphogroups that together include many of the most contentious members of the Ediacaran biota: the rangeomorphs, dickinsoniomorphs and erniettomorphs. Members of these groups have all, at some point, been interpreted as animals, with some researchers considering members of all three groups to share a self-similar body plan, perhaps indicating a common evolutionary history (Seilacher, 1989, 1992; Buss & Seilacher, 1994). We favour the use of morphogroups because it confers phylogenetic neutrality, but we note the possibility that unrelated taxa may be grouped together within such morphogroups, potentially obscuring phylogenetic signal. These concerns may be allayed by independent attempts to resolve the phylogenetic relationships among the Ediacaran grades that have provided some support for the biological reality of some morphogroups (Dececchi *et al.*, 2017). Regardless, while we acknowledge that the composition of these morphogroups may not be entirely coherent in phylogenetic terms, we consider them to provide a useful framework within which to sample the disparity of Ediacaran macro-organism body plans.

Hoyal Cuthill & Conway Morris (2017) have attempted to explain variation among Ediacaran frondose organisms as a consequence of ecophenotypism, produced in response to variation in nutrient levels in the water column across different palaeoenvironments. This suggestion potentially introduces an alternative explanation for morphological variation otherwise interpreted as taxonomic or ontogenetic. While we recognise the presence of some ecophenotypic variation within Ediacaran assemblages, we note that population-level studies of frondose organisms continue to document discrete taxonomic variation (e.g. Kenchington & Wilby, 2017). Hoyal Cuthill & Conway Morris (2017) based their study on only three, anatomically discrete, specimens, representing taxa that are known to co-occur on bedding planes (Narbonne *et al.*, 2009), consistent with morphological variation existing within assemblages subject to similar palaeoenvironmental regimes. Until relationships between morphology and ambient nutrient levels can be demonstrated we consider size variation within Ediacaran taxa to reflect ontogeny.

### (1) Rangeomorpha

Rangeomorpha (Fig. 1) encompasses organisms that share a body plan comprising one or multiple fronds constructed of serially repeated, leaf-like, self-repeating branches [see supplementary online material (SOM) of Erwin *et al.*, 2011]. Rangeomorphs were seemingly sessile organisms that lived in deep- and shallow-marine depositional environments, and are a stratigraphically long-ranging morphogroup, spanning the interval ∼570–541 Ma (Boag, Darroch, & Laflamme, 2016; Pu *et al.*, 2016). Rangeomorphs can be uniterminal (with one apparent distal terminus: e.g. *Charnia masoni*), biterminal (e.g. *Fractofusus*) or multiterminal (e.g. *Bradgatia*), and the arrangement of their branches has been proposed as a basis for distinguishing between taxa (Narbonne *et al.*, 2009; Brasier *et al.*, 2012). Morphogenesis has been considered most widely in the cosmopolitan taxon *Charnia masoni*, which possesses many features characteristic of rangeomorphs (Brasier & Antcliffe, 2004, 2009).

#### (a) Charnia masoni

*Charnia masoni* (Fig. 1E) is a uniterminal rangeomorph with a global late-Ediacaran distribution, found in the UK (e.g. Wilby *et al.*, 2015), Newfoundland (e.g. Laflamme *et al.*, 2007), northwestern Canada (Narbonne *et al.*, 2014), South Australia (e.g. Gehling & Droser, 2013), the White Sea of Russia (Fedonkin, 1990), and Siberia (e.g. Grazhdankin *et al.*, 2008). It has been variously compared to algae (Ford, 1958), fungi (Peterson, Waggoner, & Hagadorn, 2003), stem-metazoans (Budd & Jensen, 2017), pennatulacean cnidarians (Glaessner, 1984), or placed in a hypothetical non-metazoan higher order group (Seilacher, 1989, 1992). Known *Charnia masoni* specimens range from ∼1 cm (Liu *et al.*, 2012) to >65 cm (Boynton & Ford, 1995) in length, with size variants typically interpreted as different ontogenetic stages in the *Charnia* life cycle (e.g. Liu *et al.*, 2012).

*Charnia* individuals of all sizes share a similar gross morphology, possessing multiple primary branches lying

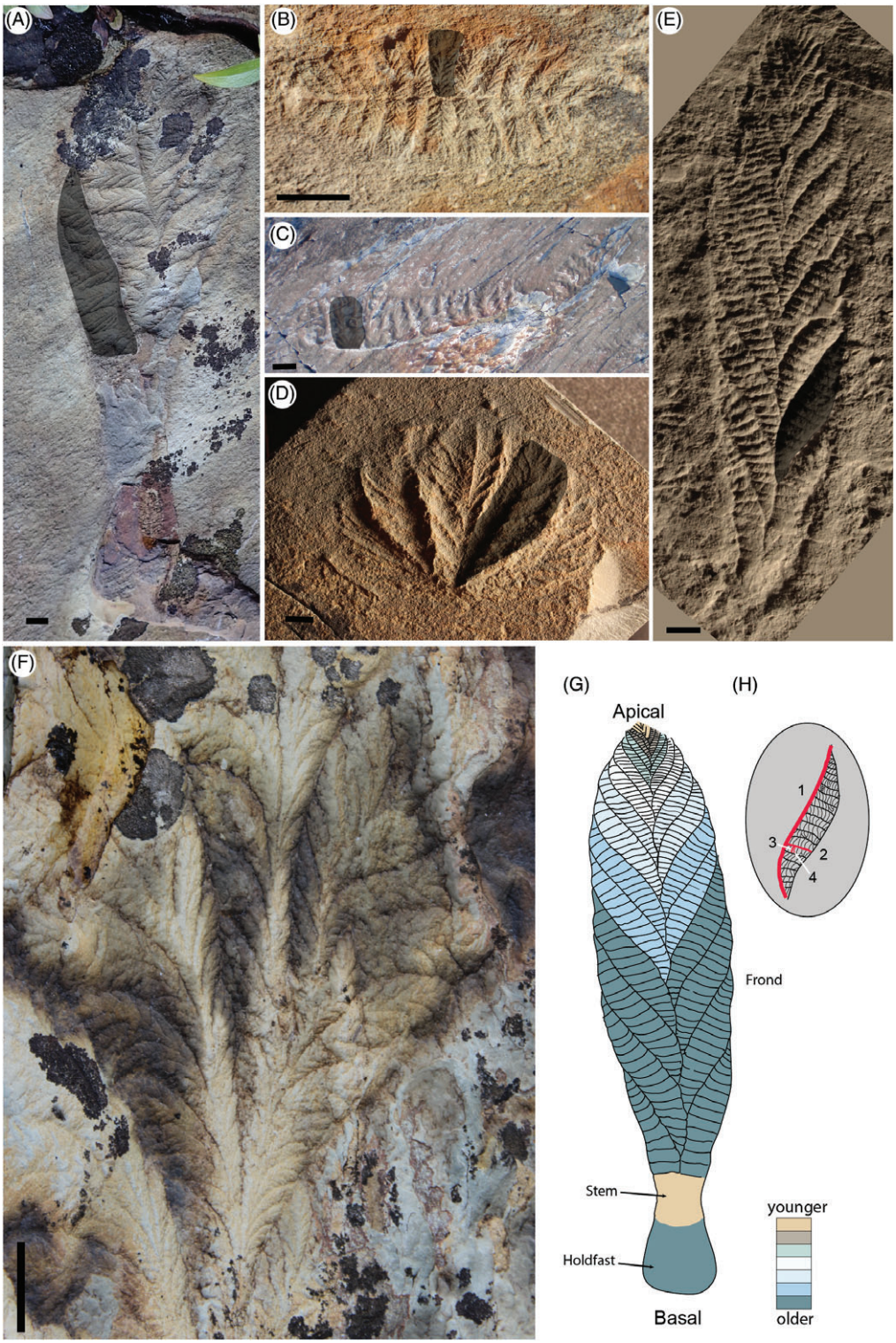


**Fig. 1.** Ediacaran rangeomorph taxa. (A) *Beothukis plumosa*, Newfoundland, Canada. (B) *Fractofusus andersoni*, Newfoundland, Canada. (C) *Pectinifrons abyssalis*, Newfoundland, Canada. (D) *Bradgatia* sp., Newfoundland, Canada. (E) *Charnia masoni*, UK. (F) Higher-order branching in an exceptionally preserved *Bradgatia* sp. specimen from Newfoundland. (G) Stylised interpretation of growth of primary branches in *Charnia masoni*. (H) The different orders of rangeomorph branches, and their arrangement within *Charnia masoni*: 1 = primary branch, 2 = secondary branch, 3 = tertiary branch and 4 = quaternary branch. Grey overlay in A–E indicates a primary branch. Scale bars: A, B, D and E = 10 mm, C = 5 cm.

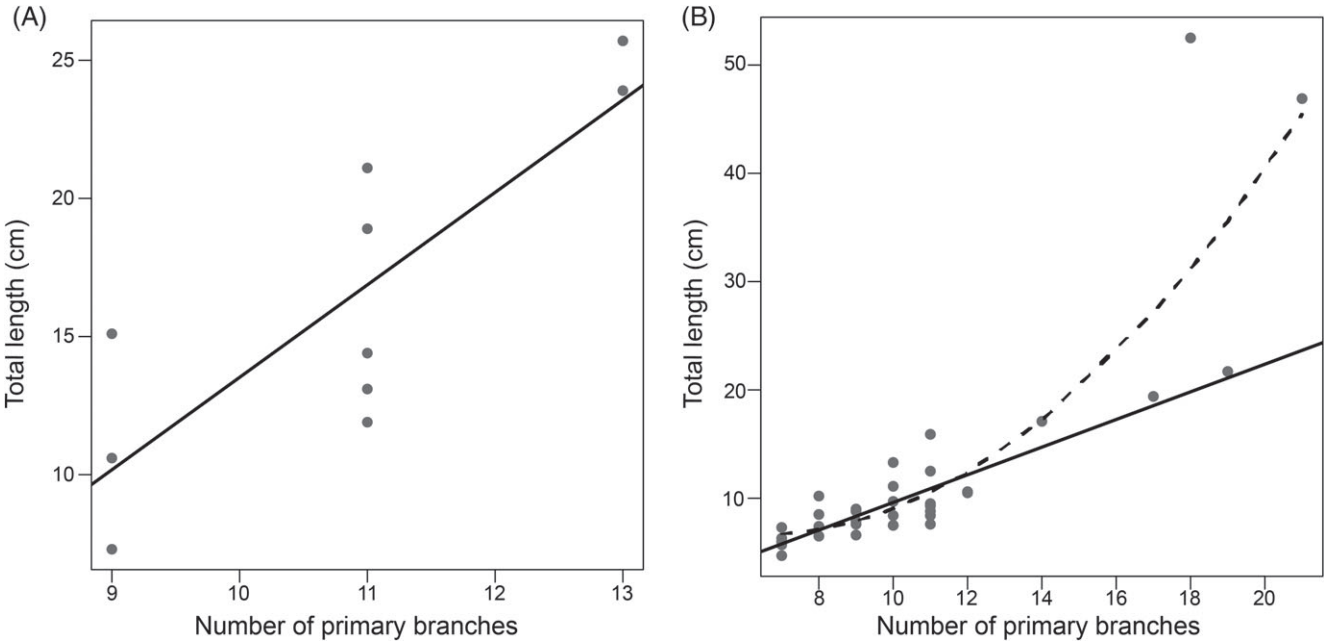


**Fig. 2.** The length of *Charnia masoni* specimens plotted against the number of primary branches in specimens from: (A) Sword Point, Newfoundland, Canada (data from Laflamme *et al.*, 2007) (data have been retrodeformed); (B) North Quarry Bed B, Charnwood Forest, Leicestershire, UK (data from Wilby *et al.*, 2015) (data were not retrodeformed). Linear models represented by solid line (fitted through a subset of data in B – excluding the two largest specimens); broken line represents a second-order polynomial model. Both populations show a linear relationship between specimen size and the number of primary branches up to specimens 49 cm in length [$P = 0.003429$ and $P = 5.327 \times 10^{-11}$ for the Laflamme *et al.* (2007) and Wilby *et al.* (2015) data sets, respectively]; specimens larger than this are not explained by a linear model [the complete Wilby *et al.* (2015) data set is best fitted by a second-order polynomial model, $P = 1.579 \times 10^{-10}$].

at a high angle along a glide plane of symmetry running through the central axis of the frond. The smallest frondose specimens appear to lack a stem, but all are considered to possess a sediment-bound holdfast to anchor them to the seafloor (see fig. 4b in Liu *et al.*, 2012). Primary branches in the smallest specimens range from five in a specimen of 1.0 cm length to seven in a specimen of 1.3 cm (Liu *et al.*, 2012). Specimens longer than ∼7 cm possess a clear but short stem, which can exhibit branching down its length (fig. 2b in Laflamme *et al.*, 2007; fig. 5.5 in Wilby *et al.*, 2015), thus distinguishing this feature from the discrete 'naked' stem (i.e. lacking branched subdivisions) of other rangeomorphs (Laflamme *et al.*, 2012) and non-rangeomorph frondose taxa (e.g. *Charniodiscus*; Laflamme, Narbonne, & Anderson, 2004). There is a broad linear relationship between the number of primary branches in *Charnia masoni* and the overall length of the organism (Fig. 2), excepting the very largest specimens, which possess proportionally fewer branches than might be expected (Wilby *et al.*, 2015). Primary branches increase in size as the organism increases in length (Wilby *et al.*, 2015). No specimens of *Charnia* have been observed to possess greater than four hierarchical orders of branching. Previously collected ontogenetic data are derived only from primary branches and so development in higher branching orders, and the number of branch orders in the smallest specimens, has yet to be discerned.

These previous observations have led to interpretation of *Charnia* as growing by the 'insertion' and subsequent inflation of branches (Wilby *et al.*, 2015). The consistent smaller size of primary branches at the apical region of individual fronds has been interpreted as evidence for a distal (apical) generative zone (Antcliffe & Brasier, 2007), with proximal primary branches (close to the holdfast) considered to have undergone a relatively longer inflation-driven period of growth (fig. 2 in Antcliffe & Brasier, 2007). The proportionally lower number of primary branches in the largest specimens could represent an ontogenetic shift from an initial 'insertion'-driven stage of growth to a second inflation-dominant interval with reduced rates of branch addition (Wilby *et al.*, 2015). The largest *Charnia* specimens have been proposed as evidence for indeterminate growth, and seem to show no upper size constraints (Wilby *et al.*, 2015).

The apparent absence of a stem in *Charnia* specimens less than ∼7 cm in length may indicate that a stem was not present in the youngest organisms (Fig. 3A, B). It is possible that the stem and holdfast were buried in small specimens, lying beneath the plane of preservation. However, these smallest specimens exhibit a 'V'-shaped termination at their base, with no suggestion of any downwards extension of the basal branches (Fig. 3A, B). If the stem was truly absent in early ontogenetic stages, emerging only later in the life cycle (Fig. 3C–E), the notion of *Charnia* possessing a single, distal growth tip (*sensu* Antcliffe & Brasier, 2007) becomes

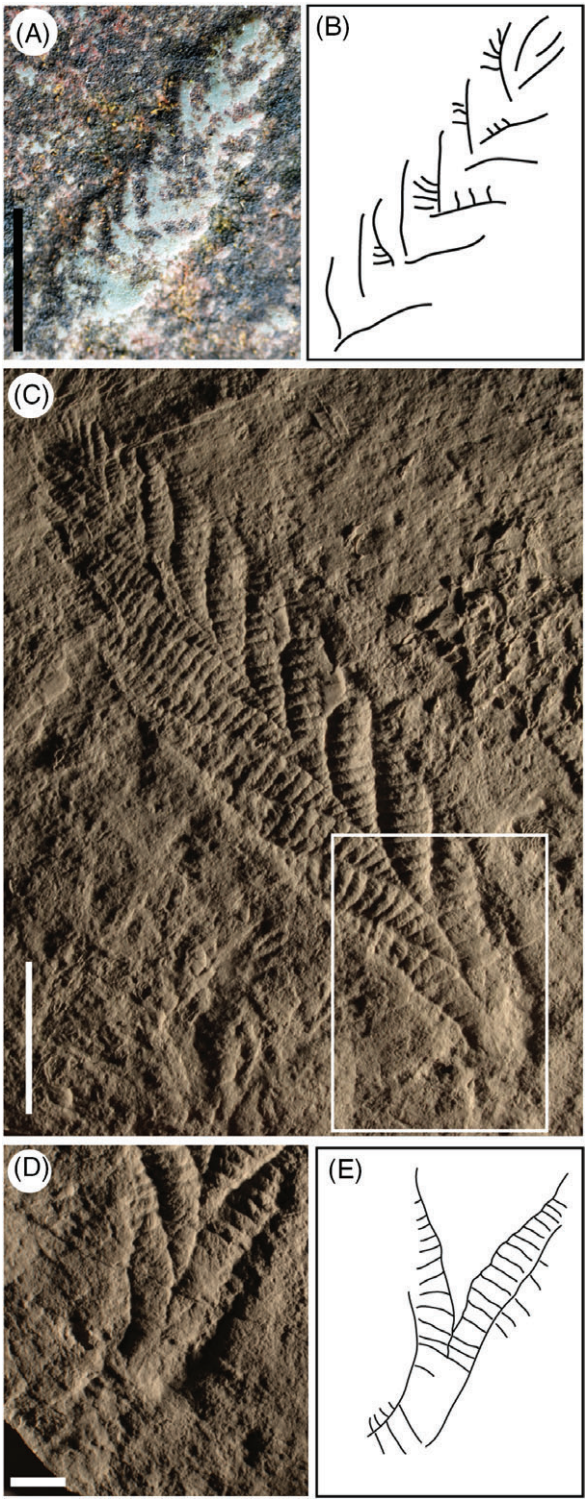


**Fig. 3.** The development of the 'stem' region in *Charnia masoni*. (A, B) *Charnia masoni* from Pigeon Cove, Mistaken Point Ecological Reserve, Newfoundland, Canada (A) and outline of specimen (B). (C) *Charnia masoni* from Charnwood Forest, Leicestershire, UK. (D, E) Stem area (enlargement of boxed region in C) (D), and in outline (E). Illustrations to second-order branch sub-division. Scale bars: A = 5 mm, C = 5 cm, D = 10 mm.

questionable since growth would also have occurred in a generative zone at the proximal end of the organism (depicted in Fig. 1G). Although *Charnia* undoubtedly possessed its smallest primary branches in the distal region of the frond (Antcliffe & Brasier, 2007), this observation alone is not proof of a solitary, distal, growth tip (see also Hoekzema *et al.*, 2017).

### (*b*) *Ontogenetic trends across the rangeomorphs*

Interpretations of growth across different rangeomorph taxa largely assume that branches underwent subdivision from a distal growth zone (Brasier & Antcliffe, 2009; Hoyal Cuthill & Conway Morris, 2014) (Table 1), and compare growth strategies across the rangeomorphs by considering inflationary growth and the appearance of new branches. In many uniterminal forms, growth appears to have proceeded in a similar way to that inferred in *Charnia* (e.g. *Trepassia wardae*; Laflamme *et al.*, 2007), but with some variation in the total number of primary branches, for example the imposition of an upper limit to the number of primary branches in certain taxa (Laflamme *et al.*, 2012; Liu *et al.*, 2016).

In contrast to *Charnia*, *Fractofusus* (Fig. 1B) does not exhibit a clear linear relationship between the size of the organism and the number (and length) of primary branches (Gehling & Narbonne, 2007). In both described species of *Fractofusus*, primary branch bundles decrease in size distally in both directions along the growth axis, implying the presence of two distal growth tips (i.e. a bipolar growth axis) if it is assumed that the smallest branches are also the youngest (Seilacher, 1989; Brasier *et al.*, 2012). *Fractofusus misrai* exhibits additional variance, with asymmetric 'subsidiary' branches emerging from between primary branches (Gehling & Narbonne, 2007).

*Bradgatia* sp. (Fig. 1D, F) from Newfoundland, Canada, is the best-studied multiterminal rangeomorph, with four known morphotypes, each considered to represent a different ontogenetic stage (fig. 3.4 in Flude & Narbonne, 2008). Primary branch lengths vary within populations from ~2 to 14 cm (fig. 8c in Flude & Narbonne, 2008), but do not appear to be tightly correlated with the morphotype-based ontogenetic sequence proposed for the taxon (Flude & Narbonne, 2008). More branches are visible in larger, and therefore, presumably, older morphotypes of *Bradgatia* (the average number increasing from four to seven across the morphs; table 1 in Flude & Narbonne, 2008). However, it may be that the more diffuse form of the larger morphotypes means that more branches are visible, rather than that new branches were 'inserted' later in life (Flude & Narbonne, 2008). Within a single primary branch, the number of secondary branches does not increase with branch length, varying between 5 and 10 in most cases (Flude & Narbonne, 2008). Two hypotheses attempt to explain how the different orders of rangeomorph branches may have grown: (*i*) fractal growth, whereby one branch order reaches a critical size, triggering the development of the next, lower, order; and (*ii*) a true inflationary model, where all branch orders are always

present and grow in concert (Flude & Narbonne, 2008). *Bradgatia* is the only rangeomorph interpreted to possess secondary growth tips, added non-deterministically at the apex of large primary branches (Brasier & Antcliffe, 2009).

In summary, rangeomorphs have been considered to grow by one of two growth models: (*i*) the 'insertion' of new units and their subsequent inflation; or (*ii*) the inflation of new units without additional 'insertion' (Table 1; Gehling & Narbonne, 2007; Bamforth *et al.*, 2008; Flude & Narbonne, 2008). *Charnia*, *Fractofusus* and *Bradgatia* all exhibit smaller primary branches in smaller specimens, and *Charnia* shows an increase in the number of primary branches over time (although such a relationship is not seen in known ontogenetic stages of all rangeomorph taxa). All rangeomorphs for which ontogeny has been considered are interpreted to have grown *via* emergence of branches either from distally located generative zones positioned at the ends of a single, central proximodistal axis (as seen in the uniterminal and biterminal rangeomorphs), or through a central axis and the production of lateral, secondary growth tips (i.e. *Bradgatia*). Although the different ontogenetic patterns described in rangeomorphs can show divergence from the pattern seen in *Charnia*, we find no developmental evidence that would preclude their inclusion within a single clade.

### (2) Dickinsoniomorpha

Dickinsoniomorpha (Fig. 4) are defined as serially repetitive organisms with anterioposterior differentiation (Erwin *et al.*, 2011 SOM), and include the genera *Dickinsonia*, *Yorgia*, *Windermeria* and *Andiva* (Erwin *et al.*, 2011). However, there is divergence of opinion concerning the composition of this morphogroup, and alternative groupings have been proposed, some of which include taxa such as *Spriggina* (Dzik & Ivantsov, 1999; Grazhdankin, 2014). Dickinsoniomorph taxa are all restricted to broadly shallow-marine settings ∼559–551 Ma (Waggoner, 2003; Boag *et al.*, 2016).

Unlike the seemingly sessile rangeomorphs, dickinsoniomorphs, specifically *Dickinsonia* and *Yorgia waggoneri*, can be associated with impressions interpreted as trace fossils, suggesting a capacity for active locomotion (Ivantsov & Malakhovskaya, 2002; Gehling *et al.*, 2005; Sperling & Vinther, 2010; although see McIlroy, Brasier, & Lang, 2009). Dickinsoniomorphs have been interpreted to exhibit evidence for internal anatomy, including gonads and diverticulae (e.g. Jenkins, 1992; Dzik, 2003), but such features have alternatively been interpreted as taphonomic artefacts (e.g. Brasier & Antcliffe, 2008). Constructional units in dickinsoniomorphs have been likened to metazoan segments (Wade, 1972), but more recent interpretations have argued that they may represent only external annulations (Sperling & Vinther, 2010), features invoked by some authors as the precursor-state to a fully metameric bauplan (Chipman, 2010). Morphogenesis has been considered most commonly in *Dickinsonia costata* (e.g. Runnegar, 1982), a taxon that has been discussed in debates surrounding the evolution of bilaterality (Malakhov, 2004; Gold *et al.*, 2015).

#### (*a*) Dickinsonia

*Dickinsonia costata* (Fig. 4D) has been described from shallow-marine siliciclastic facies in South Australia and Russia. It exhibits an approximately oval outline, with distally expanding units emanating from a visible central midline. Units are continuous across the midline (Runnegar, 1982; Gold *et al.*, 2015), imparting a bilateral symmetry. *D. costata* in Australia range from ∼6–250 mm in length (Reid *et al.*, 2017), with size variants commonly considered to represent different ontogenetic stages (e.g.Evans *et al.*, 2017 ; Hoekzema *et al.*, 2017). Smaller specimens possess fewer units (as few as 12) than larger ones (which can have as many as 74; Sperling & Vinther, 2010). A triangular, undivided region seen in small specimens encompasses a proportionally smaller area of the body in increasingly larger specimens (the deltoidal region, e.g. Hoekzema *et al.*, 2017), suggesting that in very early ontogenetic stages there may not have been any units at all (Ivantsov, 2007). The largest units are located close to the middle of the organism, not at either pole (Sperling & Vinther, 2010; Hoekzema *et al.*, 2017). The position of the smallest units has often been used to infer the position of a growth zone (Runnegar, 1982; Ivantsov, 2007; Evans *et al.*, 2017), which has been described as being in a 'posterior' position (Ivantsov, 2007) with units added terminally (Gold *et al.*, 2015; Evans *et al.*, 2017). Gold *et al.* (2015) follow Jacobs *et al.* (2005) in their definition of 'terminal addition', but figure a truly terminal generative zone (fig. 2 in Gold *et al.*, 2015). Evans *et al.* (2017) do not define 'terminal addition', but reference Gold *et al.* (2015) and so we assume they also follow the definition of 'terminal addition' in Jacobs *et al.* (2005). However, recent work suggests that *Dickinsonia* instead added units at the opposing pole (Hoekzema *et al.*, 2017). The latter authors characterise growth of units within populations of organisms interpreted to represent multiple ontogenetic stages, and present evidence for differentiation of new units from the margins of the undifferentiated region itself. In this scenario, which we support, the generative zone of *Dickinsonia* may be considered pre-terminal (Fig. 4F). Further recent work has considered *Dickinsonia costata* to represent a paedomorphic variant of *Dickinsonia tenuis* (which possesses a greater unit count than *D. costata*; Zakrevskaya & Ivantsov, 2017).

These observations together suggest that *Dickinsonia* grew by the 'insertion' of new units, which then underwent subsequent inflation (see Runnegar, 1982; Fig. 4F). Larger specimens possess proportionally fewer units relative to their length, implying a reduction in the rate of unit addition (Evans *et al.*, 2017; Hoekzema *et al.*, 2017). However, there is variation in the number of units per specimen that is seemingly independent of (active?) contraction noted in many individuals (Evans *et al.*, 2017). *Dickinsonia* has been conflictingly interpreted to show both a pre-determined (Runnegar, 1982; Ivantsov, 2007) and an indeterminate (Retallack, 2007) mode of growth, but the apparent absence of size outliers belonging to *D. costata* appears to suggest that deterministic growth is more likely. The species *Dickinsonia rex*, however, could reach much greater sizes (∼43 cm; Jenkins,

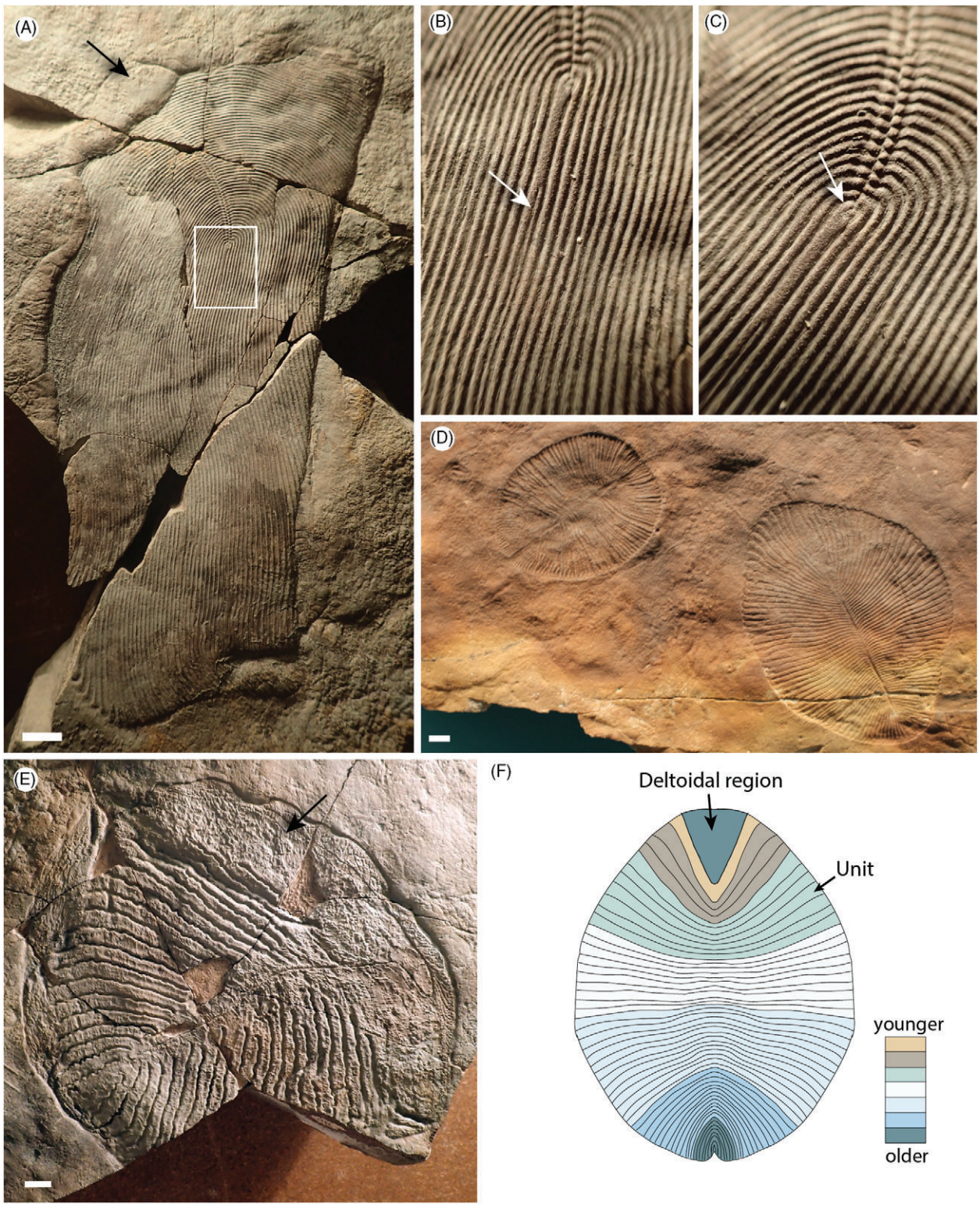


**Fig. 4.** Ediacaran dickinsoniomorph taxa. (A) *Andiva ivantsovi*, White Sea, Russia. [Palaeontological Institute Moscow (PIN) specimen number 3993–5623]. (B, C) Enlargements of the boxed area in A. The areas of unit differentiation are indicated by white arrows, and undivided regions on *Andiva* and *Yorgia* are indicated by black arrows. (D) *Dickinsonia costata*, South Australia [South Australia Museum (SAM) specimen numbers P49354 and P49355]. (E) *Yorgia waggoneri*, White Sea, Russia (Holotype PIN 3993–5024). (F) Stylised interpretation of growth of *Dickinsonia costata*, following the growth model proposed in Hoekzema *et al.* (2017). Scale bars = 10 mm.

1992), suggesting that a determinate pattern of growth cannot yet be assumed for all *Dickinsonia* species.

### (*b*) *Ontogenetic trends across dickinsoniomorphs*

Unlike *Dickinsonia*, *Andiva ivantsovi* (Fig. 4A–C) is not bilaterally symmetrical, bearing a glide plane of symmetry along its axial midline. *Andiva* does possess an undivided region, but whereas in *Dickinsonia* this region appears to diminish in size as the organism grew, its proportions relative to the overall organism are seemingly maintained in *Andiva* (Fedonkin, 2002). *Andiva* differs from *Dickinsonia* in several other regards. For example, there is seemingly no clear relationship between specimen size and number of units. Like *Andiva*, *Yorgia waggoneri* (Fig. 4E) also appears to possess an undivided region at all known stages of growth (Dzik & Ivantsov, 1999; Ivantsov, 2007). The smallest *Yorgia* specimens possess 10–12 independent units, while larger specimens can have up to 70 (i.e. 35 'isomer pairs'; Ivantsov & Fedonkin, 2001) aligned along a glide plane of symmetry, *contra Dickinsonia*. If *Dickinsonia*, *Andiva* and *Yorgia* are closely related, it is fair to assume they would possess a similarly positioned generative zone. We find potential evidence that *Andiva* differentiated units from the opposite end to its undifferentiated area (i.e. its anti-deltoidal pole, see Hoekzema *et al.*, 2017), based on the recognition of an apparently partially differentiated unit (Fig. 4A–C). While this could be alternatively interpreted as two overlying units, if correct this observation suggests that in *Andiva*, differentiation occurred at a truly terminal generative zone, at the opposite end to the non-differentiated region of the organism. Further work on a greater number of specimens is required, but it seems that the morphological differences previously outlined between *Dickinsonia* (bilaterally symmetrical with a proportionally variable deltoidal area) and *Andiva* (glide symmetry, and an undifferentiated crescentic region of fixed size relative to the body) may be corroborated by developmental differences, with growth progressing at different ends of the organisms with respect to their undifferentiated regions. Whether the undifferentiated deltoidal region of *Dickinsonia* and the crescentic region of *Andiva* are homologous remains to be determined. Our developmental comparisons do, however, raise the possibility that while *Dickinsonia* is arguably of the same morphological grade as other 'dickinsoniomorph' taxa, it may not ultimately belong to the same clade.

## (3) Erniettomorpha

Erniettomorphs (Fig. 5) are defined as serially repetitive organisms constructed entirely of tubular units arranged into fronds, 'sac-like' or 'canoe-like' benthic recliners, or flat-lying mats (SOM of Erwin *et al.*, 2011); this definition clearly encompasses a broad range of morphologies. Erniettomorphs are prominent constituents of the latest Ediacaran macrofossil assemblages of Namibia (~550–541 Ma) (Darroch *et al.*, 2015; Boag *et al.*, 2016), and Nevada (Smith *et al.*, 2017), yet their biology is little understood. Only two taxa, *Ernietta plateauensis* (a sac-like form) and *Pteridinium simplex* (a canoe-like form), have undergone recent detailed study (Elliott *et al.*, 2011, 2016; Ivantsov *et al.*, 2016). *Pteridinium simplex* is the most widely studied erniettomorph from an ontogenetic perspective, but whether its growth strategy is broadly applicable to all erniettomorphs is debatable given the morphological disparity of this group.

### (*a*) Pteridinium simplex

*Pteridinium simplex* (Fig. 5A, B) appears to have been constructed of three vanes of tubular units (Fig. 5B) that meet in an alternating fashion at a central 'seam', imparting a glide plane of symmetry (Grazhdankin & Seilacher, 2002; Meyer *et al.*, 2014). Complete specimens range from 6.0 cm in length (along the central seam, displaying 26 units) to 19.2 cm (with 55 units) (Grazhdankin & Seilacher, 2002). The number and length (long axis) of individual units appears to correlate linearly with the organism's total length, but the height of the organism (the distance between the central seam and the termination of the long axis of the units) does not follow a similar relationship (Grazhdankin & Seilacher, 2002). The relationship between unit length and overall length reveals two distinct morphological groupings of *Pteridinium*; one showing a positive correlation between the two variables, and one showing no correlation (Grazhdankin & Seilacher, 2002). This ontogenetic variation may imply the presence of two distinct *Pteridinium* species, or may alternatively hint at ecophenotypic variation within the taxon (the study of which amongst the Ediacaran macrobiota remains in its infancy: Kenchington & Wilby, 2017; Hoyal Cuthill & Conway Morris, 2017).

Specimens of *Pteridinium* can taper at one or both ends, with the tapering tip previously inferred to be the growth tip (Grazhdankin & Seilacher, 2002; Laflamme, Xiao, & Kowalewski, 2009). *Pteridinium* has thus been variously considered as both unipolar (Grazhdankin & Seilacher, 2002) and bipolar (Laflamme *et al.*, 2009), although the lack of a tapering tip in some specimens may reflect a taphonomic bias (Seilacher, 1989). The distal-most unit can be positioned on either side of the central seam, suggesting that *Pteridinium* added units sequentially across its different vanes (Tojo *et al.*, 2007; although see Laflamme *et al.*, 2009). *Pteridinium* has previously been considered to grow mainly by the 'insertion' of new units over time (Laflamme *et al.*, 2009), but it appears that one morph also grew by the observable inflation of pre-existing units (Grazhdankin & Seilacher, 2002). Specimens that are ~6 cm long have been inferred to be immature (Grazhdankin & Seilacher, 2002), but there are no documented specimens of comparable size to those of the smallest rangeomorphs and dickinsoniomorphs (i.e. 10 mm or less).

### (*b*) *Ontogenetic trends across the erniettomorphs*

The only other erniettomorph for which there is sufficient data to deduce ontogenetic information is *Ernietta plateauensis* (Fig. 5C). Unlike *Pteridinium*, the number of units remains

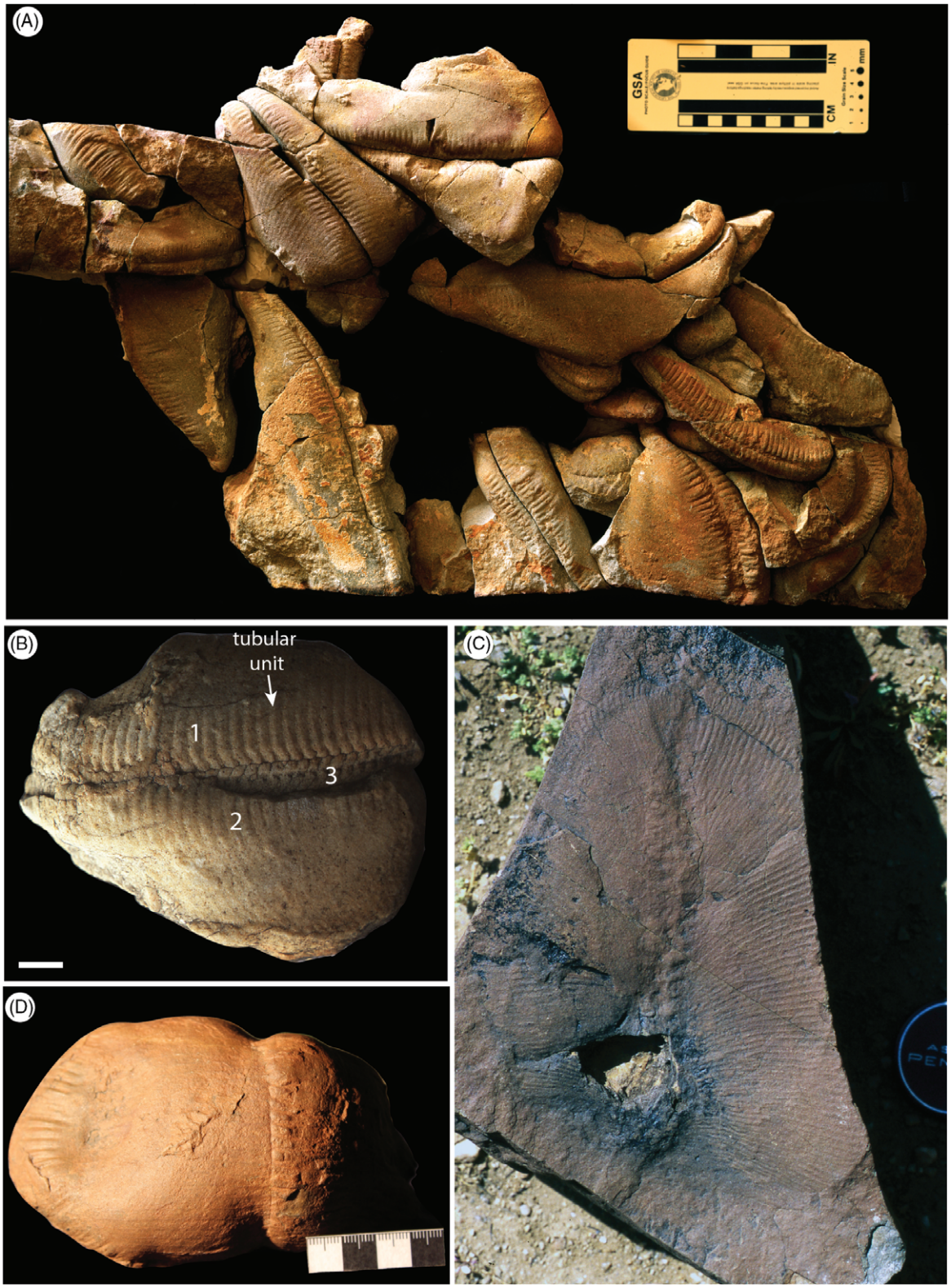


**Fig. 5.** Ediacaran erniettomorph taxa. (A, B) *Pteridinium simplex*, Namibia. Numbers identifying the three identified vanes. (C) *Swartpuntia germsii*, Namibia. (D) *Ernietta plateauensis*, Namibia. Scale bars = 10 mm. Images courtesy of D. Grazhdankin (A and B from Grazhdankin & Seilacher, 2002), M.D. Brasier (C), and M. Laflamme (D).

relatively constant (23–28 on either side of the organism) across specimens of 35–55 mm in basal width (known size range 30–80 mm in width; Bouougri *et al.*, 2011). This suggests that growth took place primarily by the inflation of units, rather than by their continued insertion, at least in larger specimens (Ivantsov *et al.*, 2016). However, there has been considerable debate as to what constitutes a 'juvenile' *Ernietta* (Hahn & Pflug, 1985; Runnegar, 1992; Schopf & Klein, 1992; Elliott *et al.*, 2016), and so we refrain from presenting an ontogenetic analysis of this taxon. Other erniettomorph taxa, such as *Swartpuntia* (Fig. 5D) (Narbonne, Saylor, & Grotzinger, 1997), have received relatively little attention in terms of their morphogenesis. Before the morphogenesis of erniettomorphs can be reliably assessed, a re-evaluation of what constitutes membership of this group is required. Consequently, it is currently not possible to compare ontogenetic processes between the erniettomorphs, and thus evaluate the utility of this morphogroup.

## IV. DEVELOPMENTAL COMPARISONS AND PHYLOGENETIC INFERENCE

### (1) Extant taxa

Among the eukaryotes, serial repetitive growth is known in the chlorophyte, streptophyte, rhodophyte, and phaeophyte algae, land plants, fungi, and members of the Metazoa (Gold *et al.*, 2015). However, the processes by which these groups attain their essentially similar morphologies are very different. Plants and algae (red, green and brown) possess apical meristems, with the repeated re-specification of lateral organs along their length (Kuhlemeier, 2007). Each lateral organ displays developmental independence and, as such, these groups are classified as modular, displaying parallel modular growth, which results in an indeterminate morphology (Kaandorp, 2012; Fig. 6A–B). Brown algae, unlike plants and other algal groups that possess only one axial growth zone (Fig. 6C), can possess multiple axial growth zones located more basally (intercalary meristems: Charrier, le Bail, & de Reviers, 2012; Fig. 6D). Brown algal intercalary meristems have been interpreted as derived, whereas the apical meristem is considered plesiomorphic (Charrier *et al.*, 2012).

Fungi are also modular and grow from the tips of hyphae (Brand & Gow, 2009), but unlike the plants and the algae they lack a truly organismal body axis. Hyphae come together to form a fruiting body, rather than modules developing from a central structure as in plants. Moreover, fungi do not exhibit differentiation of new units over time. The fruiting body emerges following the formation of a 'hyphal knot' by multiply branched hyphae, and subsequently differentiates into the constituent parts (e.g. in the button mushroom *Agaricus bisporus*; Umar & Van Griensven, 1997).

While not serially repetitive, since a lichen affinity has been advanced for members of the Vendobionta (Retallack, 1994), their morphogenesis must be considered. Lichens are known to exhibit an indeterminate form, and so display

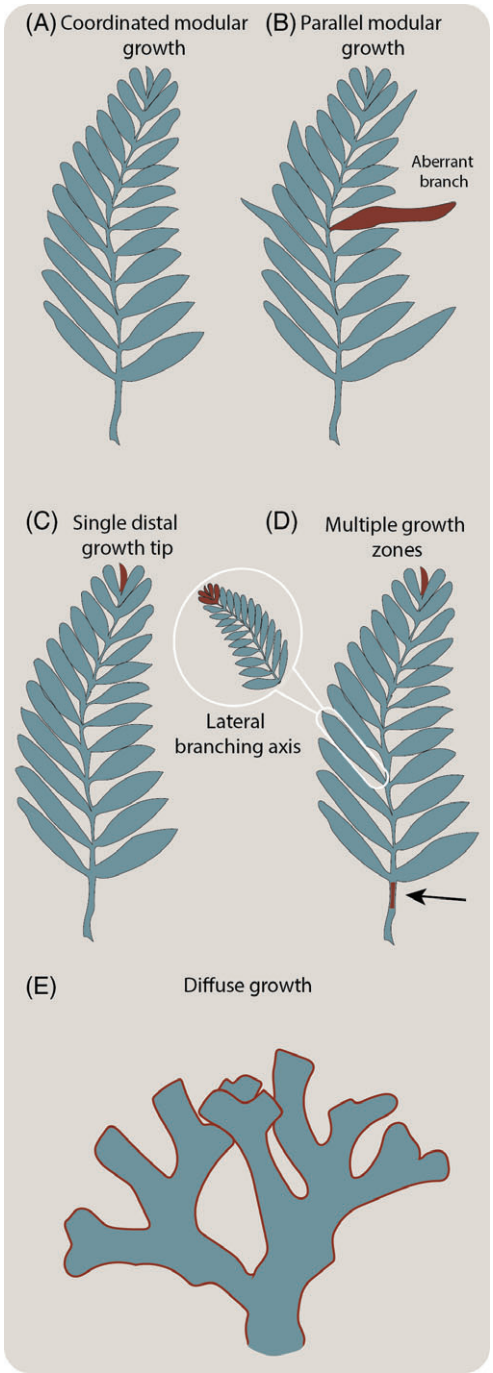


**Fig. 6.** Schematic diagram showing the forms of growth observed in extant clades with serial repetition of component units; red indicates the style/feature of growth discussed. (A) Coordinated modular growth, seen in certain metazoan groups. (B) Parallel modular growth, common in plants and red, green and brown algae, with an aberrant branch highlighted in red. (C, D) Positioning of different central (additional growth zone highlighted with black arrow) and lateral growth zones/tips in extant serially repetitive groups. Single apical axes are seen in green and red algal groups, whereas multiple axes are seen in various metazoan and brown-algal groups. (E) Diffuse growth, as seen in colonial bilaterian groups characterised by colony-wide tip growth.

parallel modular growth (e.g. fig. 1 in Suetina & Glotov, 2010).

Serial repetition is achieved in plants and algae by the presence of a totipotent meristem (a zone of cell proliferation that gives rise to the organs and tissues of a plant), but in colonial animals it can be achieved in a number of different ways. Within Cnidaria, coloniality is widespread in the anthozoans and the hydrozoans, and with two main mechanisms of colonial growth at play. Monopodial growth is much like the meristematic growth seen in plants, whereby growth proceeds primarily from an (sub)apical growth tip; in athectate hydrozoans, lateral branches are specified successively and these then display monopodial growth themselves. In thectate hydroids, this same pattern of monopodial growth cannot occur due to the presence of the theca. In these forms, the apical stem tip acts in a fashion similar to a meristem, specifying new lateral shoots on both sides of the organism simultaneously (Berking, 2006). Sympodial growth involves the cessation of growth at the apical growth tip, and the re-specification of the 'apex' as outgrowths from successive lateral growth tips (Berking, 2006). Both monopodial and sympodial growth can occur either separately or concurrently. Some colonial anthozoans do not exhibit classical monopodial growth, with new branches emerging from a basal and pre-terminal growth zone in Pennatulacea (Antcliffe & Brasier, 2007). Colonial cnidarians are also known to show colony polymorphism (discontinuous variation in zooid morphology within colonies: Hyman, 1940*a*; Boardman, Cheetham, & Oliver, 1973). In such cnidarians, repeated units tend to appear in sets, or whorls (Gold *et al.*, 2015).

Extant members of Porifera do not show a serially repetitive body plan in the same way as certain cnidarians, and do not display the same level of colonial integration (i.e. the division of labour). However, certain sponges (e.g. the demosponge *Callyspongia vaginalis*) are constructed of serially repeated units. Recent work has elucidated a broad repertoire of developmental regulatory genes in the Porifera, hinting at ancestral complexity in the early sponges (Leininger *et al.*, 2014). While Placozoa has been considered sister to Bilateria (Collins, 1998), recent work suggests that the cnidarians are sister to Bilateria (e.g. Cannon *et al.*, 2016). No-one has yet reconstructed the ancestral states of Placozoa (or Ctenophora for that matter), and the presumably simplified morphology of extant placozoans, and the derived nature of extant ctenophores, means we should not exclude either group from the Ediacaran debate.

Many colonial bilaterians (belonging to Rouphozoa and Gnathifera; Laumer *et al.*, 2015) tend to show, in the broadest sense, a more diffuse form of colonial growth (Fig. 6E). In bryozoans, which can possess frondose or arborescent forms, new zooids emerge by budding, with the pattern of budding being almost species specific and determining the form of the colony (Hyman, 1940*b*). The entoprocts, once considered to be members of Bryozoa, are largely colonial in form. Rather than taking an arborescent form, entoprocts often grow through laterally spreading stolons, with vertically projecting zooids emerging at intervals. Meanwhile the rotifers display an aggregative form of colonialism, whereby juveniles become tangled up and eventually adhere to each other by production of an adhesive string from a foot gland (Surface, 1906).

The serially repetitive structures observed in members of the segmented unitary Bilateria – the arthropods, annelids and chordates – develop largely through the process of posterior growth *via* the specification of units in parallel with the elongation of the anterior–posterior axis (Jacobs *et al.*, 2005). Whereas in many serially repetitive organisms there is a disjunct between the growth of individual units and the growth of the main body axis, the two are concurrent in the segmented Bilateria. The specification of units is sequential in most of these bilaterians, but there are exceptions, such as the long-germ-band insects (e.g. *Drosophila melanogaster*), which specify the entire anterior–posterior axis simultaneously (Liu & Kaufman, 2005). The patterns imparted by different forms of segmentation can manifest in different ways. Organisms can be homonomously segmented, whereby segments are largely identical, or groups of segments performing similar tasks may group together into functional units known as tagmata.

### (2) Implications for the Ediacaran macrobiota

Proposed members of the rangeomorphs, dickinsoniomorphs and erniettomorphs have all been described as growing by either the differentiation of new units, the inflation of pre-existing units (at known ontogenetic stages), or a combination of the two (Table 1). Description of growth by the differentiation of new units and/or their subsequent expansion alone is, however, uninformative for constraining phylogenetic affinity, since this method of formulating new units is universal among multicellular eukaryotic groups (Bonner, 1952). The absence of data on the very earliest growth stages (of a few millimetres or less) in Ediacaran taxa also hampers efforts to determine the point at which differentiation occurred in the life cycle in some taxa.

The position of the generative zone is potentially a more useful developmental character, but identification of this trait in rangeomorphs, dickinsoniomorphs, and erniettomorphs remains difficult since the assumption that the position of the smallest units correlates with the position of the generative zone has recently been questioned (Hoekzema *et al.*, 2017). In the following discussion, we assume that previously ascribed generative zones as discussed in the above sections are correct, but note that such assumptions remain unproven.

Rangeomorphs exhibit a non-deviant form (i.e. aberrant-length branches have not been observed in thousands of studied specimens). It is, therefore, highly likely that rangeomorphs do not exhibit the parallel modular growth characteristic of non-metazoan serially repetitive groups. Their shape is seemingly constrained at both the organismal level, and at the level of individual branches (including subsidiary branches; Gehling & Narbonne, 2007), across the known ontogenetic series.

Unlike Fungi, rangeomorphs exhibit the differentiation of new units. The presence of a basal growth zone (in the stem and potentially in some of the lowermost primary branches), as well as an apical one, at least in *Charnia*, would ally them to Eumetazoa (but of course our understanding of plesiomorphic states in early diverging metazoans is wanting). The presence of discrete (as opposed to diffuse) growth tips would argue against affinities with most members of Rouphozoa and Gnathifera, but the likely presence of multiple axial growth zones (in *Charnia*) and potential secondary growth tips (in *Bradgatia*), is reconcilable with known variation in members of the colonial cnidarians. Based on current data, we cannot rule out a stem-metazoan affinity for rangeomorphs (if Porifera are the sister lineage to all other metazoans; Pisani *et al*., 2015), or, indeed, a stem-poriferan affinity, but the general paucity, as opposed to conflict, of data prevents further assessment (Fig. 7). We do not consider a ctenophore affinity likely since both extant ctenophores and organisms considered to be stem-ctenophores, including the Ediacaran *Eoandromeda*, are considered to be motile (Tang *et al*., 2011).

Dickinsoniomorphs as currently defined also lack evidence of parallel modularity, and show the differentiation of new units across ontogeny, precluding algal and fungal phylogenetic affinities. When combined with trace fossil evidence for motility, and anatomical evidence (Sperling & Vinther, 2010), this developmental constraint likely requires that they are metazoan. The data of Hoekzema *et al*. (2017) suggest that *Dickinsonia* may have possessed a pre-terminal growth zone along with concurrent inflative growth in lateral units and the main growth axis, which can be reconciled with the basal and pre-terminal generative zone of extant segmented bilaterians (Fig. 7A). There are, of course, exceptions to this rule, such as Onychophora (which grow from a true terminus; Anderson, 1973), or Nematoida (where a secondary loss of serially repetitive units makes confirmation of a pre-terminal growth zone difficult), but these conditions have been considered to be derived from an ancestral pattern of pre-terminal addition (Jacobs *et al*., 2005). The monopodial serially repetitive cnidarians also show a pre-terminal mode of extension rather than a true terminal growth zone, so a pre-terminal generative zone for *Dickinsonia* remains compatible with such organisms. However, organisms of cnidarian grade may also exhibit truly terminal differentiation (e.g. monopodially growing athectate hydrozoans; Berking, 2006). A placozoan affinity for *Dickinsonia* (Sperling & Vinther, 2010) is difficult to evaluate on developmental grounds given the low diversity and disparity of extant placozoans, and remains a viable possibility (Fig. 7). The potential for a truly terminal growth zone in *Andiva* (Fig. 7) could, however, suggest that a non-bilaterian affinity is possible for at least some dickinsoniomorph taxa.

Currently, the erniettomorphs are too poorly understood to infer their phylogenetic position from developmental data. Members of Erniettomorpha have been considered to show morphological similarities to members of the annulated *Dickinsonia*-like taxa (e.g. Budd & Jensen, 2017), but whether this evidences a phylogenetic relationship is unclear. The relative consistency of overall form in erniettomorphs suggests that they do not exhibit parallel modular growth and, thus, they are unlikely to be plants or algae. Continuous differentiation of new units in *Pteridinium* seemingly rules out a fungal affinity. There are no current data to exclude *Pteridinium* from Metazoa, but there is similarly no additional evidence to support a metazoan affinity. Given our poor knowledge of erniettomorphs, we cannot currently extrapolate from *Pteridinium* to other organisms. Indeed, this review has highlighted significant gaps in knowledge of development in multiple Ediacaran taxa, as well as taxonomic issues that require resolution before morphogenesis can be meaningfully addressed in other morphogroups.

## V. IMPLICATIONS FOR DEVELOPMENTAL EVOLUTION

Developmental evidence supports a metazoan affinity for rangeomorphs (Fig. 7B). Their multiple axial growth zones, as well as their asymmetric glide plane of symmetry, apparent in all known life stages, argue against most bilaterian affiliations, but we note that forms of glide symmetry are known in bilaterian taxa including echinoids (e.g. between plates in the interambulacral zone) and graptolites (e.g. *Eoglyptograptus*). There are also rare reports of bilateral symmetry at higher branching orders in some rangeomorphs (figs 3D, 4A, 5C in Flude & Narbonne, 2008), potentially revealing complexity in the axial patterning of these organisms, and illustrating that symmetry may not represent a reliable phylogenetic indicator for Ediacaran taxa.

The rangeomorphs appear to have one main body axis and one lateral branching axis, an arrangement very similar to various cnidarian organisms (Watanabe *et al*., 2014), with which they also share developmental similarities, i.e. a conserved form and potential positioning of the generative zone. The possibility that rangeomorphs possessed a third body axis (akin to the dorso-ventral axis), cannot yet be excluded, but seems unlikely given evidence to suggest that some rangeomorphs were identical on both 'sides' (e.g. fig. 3 in Seilacher, 1992; fig. 5.2 in Wilby *et al*., 2015; although see Gehling & Narbonne, 2007, for a discussion of taphonomic reasons for why a third vane may not be preserved in *Fractofusus*). Sponges are conventionally interpreted to possess just one principal body axis, but a reduction in the number of body axes may be a consequence of simplification (e.g. Ferrier, 2015). Therefore, resolution of the rangeomorphs as falling within the metazoan stem or, indeed, total-group Porifera, cannot be excluded.

The rangeomorphs do not show either true radial symmetry or bilateral symmetry, but the possibility that rangeomorphs like *Charnia* displayed biradial symmetry could prove informative. If the rangeomorphs belong to the eumetazoan stem, their possible possession of biradial symmetry could support the notion that biradiality was a

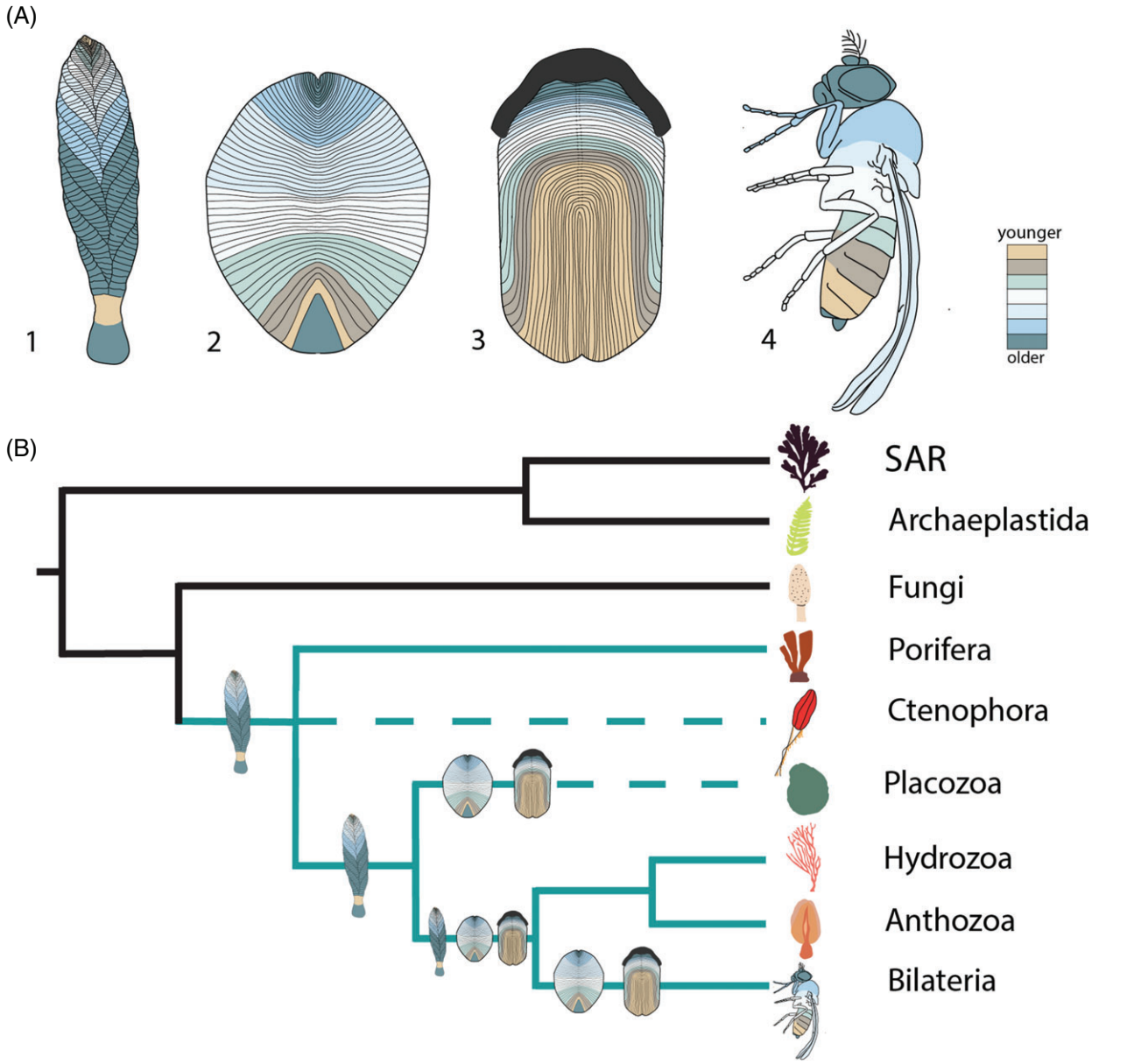


**Fig. 7.** (A) Interpretive growth models of: 1, *Charnia masoni*; 2, *Dickinsonia costata*; 3, *Andiva ivantsovi*; 4, an extant bilaterian comparator. (B) A simplified eukaryote phylogeny including only groups with serially repetitive body plans to which the Ediacaran morphogroups have been compared. SAR = Stramenopiles, Alveolates and Rhizaria. The suggested phylogenetic positions of *Charnia*, *Dickinsonia* and *Andiva* are presented as discussed in the text (we include *Andiva* as possibly being resolved within the Bilateria because although our morphological data may suggest a truly terminal generative zone, this is based on one specimen and additional data are required to confirm or refute this). Green represents metazoan lineages. Dashed lines indicate the possible position of a group (owing to uncertainty surrounding the phylogeny of the basal Metazoa; e.g. Dunn *et al.*, 2014).

precursor to bilateral symmetry in metazoans (Martindale & Henry, 1998). This is particularly pertinent given that the rangeomorphs may themselves have possessed bilateral symmetry at smaller branch orders (Flude & Narbonne, 2008). Alternatively, tentative biradial symmetry could support the idea that early metazoans experimented with variants of radial symmetry independent of phylogeny (see also the putative stem-ctenophore *Eoandromeda* which exhibits octoradial symmetry, the triradial form *Tribachidium*, tetraradial *Conomedusites*, and pentaradial *Arkarua*; Xiao & Laflamme, 2009).

*Dickinsonia*, like rangeomorphs, appears to possess one major body axis and one lateral axis, with insufficient evidence to determine differentiation across a third axis [although see Evans *et al.* (2017) for discussion of *Dickinsonia* 'height']. We resolve *Dickinsonia* as a member of total-group

Metazoa (Fig. 7B), likely within the Placozoa plus Eumetazoa total group, on the basis of the developmental evidence presented above, combined with the apparent capacity for active locomotion (see Hoekzema *et al.*, 2017).

Consideration of *Eoandromeda octobrachiata* as a stem-ctenophore (Tang *et al.*, 2011) has resulted in attempts to find homology between the body axes of radial and non-radial Ediacaran taxa. The asymmetric head region of *Yorgia* has been speculatively likened to two of the three branch-like structures that make up *Tribrachidium* (Budd & Jensen, 2017), implying axial homology between the dorso-ventral axis of *Tribachidium* and the 'anteroposterior' axis of dickinsoniomorphs. In the absence of an asymmetric undivided region in some dickinsoniomorphs, and even in some *Dickinsonia* specimens, we do not consider that there are sufficient grounds to consider these axes to be homologous.

If members of the Dickinsoniomorpha can be resolved with bilaterians, they may prove informative on the appearance of bilaterian characters. In the evolution of metamerism, a determinate form (i.e. a pre-determined number of units) likely appeared late; well after the initial appearance of true metamerism (Vroomans, Hogeweg, & Tusscher, 2016). In *Dickinsonia*, organisms of different sizes display variable numbers of units, such that the number of units does not appear pre-determined (Evans *et al.*, 2017; Hoekzema *et al.*, 2017). Therefore, if *Dickinsonia* was truly metameric (and future work is required to establish this), the fossil data would appear to concur with these prior theoretical predictions. Interestingly, the positions of putative internal anatomical structures preserved within *Dickinsonia* (e.g. Dzik & Ivantsov, 2002; Zhang & Reitner, 2006) do not correlate with the positions of the visible units considered to be on the exterior of the organism. As such, if these structures represent true biological features, and these organisms were truly segmented, they must have been heteronomously so (i.e. where segments are non-identical), possessing tagmata. While it is likely that the three main segmented bilaterian groups all developed segmentation independently of each other, it appears that the homonomous state is plesiomorphic to the arthropods and annelids (being present in the stem-lineages of these clades if we discount highly derived tagma in the head regions; e.g. Parry, Vinther, & Edgecombe, 2015; Ortega-Hernández, Janssen, & Budd, 2016), whereas heteronomous segmentation appears plesiomorphic to the vertebrates [for example, in the vertebral column (Jacobs *et al.*, 2005)]. We therefore find that dickinsoniomorphs do not sit comfortably in the stem lineages of annelids or arthropods on account of their seemingly heteronomous state. However, the absence of any chordate diagnostic characters means they cannot be reconciled with chordates either. Therefore, if members of Dickinsoniomorpha are resolved as being segmented, in this scenario we consider it most likely that they represent a bilaterian group that independently adopted a segmented form.

Another consideration is that some dickinsoniomorphs (perhaps most notably *Yorgia*) exhibit glide symmetry, not bilateral symmetry, meaning that under the scenario in which the dickinsoniomorphs do represent a coherent clade, any 'segments' would be discontinuous across the midline. Two possibilities then arise: *Yorgia* is not segmented, but does possess external annulations that may or may not be a precursor state to true segmentation; or conversely, *Yorgia* does display a form of derived segmentation similar to that seen in long-germ-band insects today, where the 'segments' are not the fundamental unit. In these cases, parasegments cross segment boundaries (Martinez-Arias & Lawrence, 1985), and pattern the embryo of certain insects (e.g. *Drosophila*).

The resolution of these organisms as falling within Metazoa does not in itself help us to resolve between their potential body axes. It is broadly true that sponges have one main body axis, diploblasts have two and triploblasts have three, and that these main axes are patterned by the same pathways and gradients, and so may be homologous (e.g. Leininger *et al.*, 2014). Wingless-related integration site (Wnt) patterning across both the oral–aboral and anterior–posterior axes (e.g. Holstein, 2012) may suggest that the primary axis across Eumetazoa is homologous, and similar Wnt patterning across the primary body axis of sponges suggests that the primary body axis across all Metazoa may be homologous (Leininger *et al.*, 2014). Similarly, bone morphogenetic protein (BMP) signalling across the directive and dorso-ventral axes (Matus *et al.*, 2006; Genikhovich *et al.*, 2015) may or may not suggest homology across Eumetazoa. However, many animal groups show major shifts in axial patterning, and so using morphology alone can lead to difficulty in identifying even analogous axes (e.g. the secondary acquisition of a pentameral body plan in starfish and sea urchins confounds identification of the anterior–posterior axis). Cnidarians, as a group, are almost typified by a number of excursions into radial symmetry (perhaps from a bilateral ancestor; Dzik, Baliński, & Sun, 2017), making the directive axis hard to identify from morphology alone. There are also examples of organisation along the dorso-ventral axis being inverted between arthropods and vertebrates [i.e. the reversal of positioning of the nerve cord (e.g. Denes *et al.*, 2007)]. Many Ediacaran macro-organisms inferred to represent ancient animals are themselves characterised by excursions into forms of radial symmetry, potentially independent of phylogeny, making points of homology difficult to ascertain. If axis homology can be proven by resolution of phylogenetic placement, these fossils could be interpreted to represent a primitive diversity of body plans, perhaps suggesting that successive disruptions and alterations to the planes of these body axes may be plesiomorphic. However, these data also warn of the problems of inferring homology across the body axes of diploblasts and triploblasts; if *Dickinsonia* is resolved as being a placozoan, or cnidarian, then definition of its main body axis as anterior–posterior (e.g. SOM of Erwin *et al.*, 2011) is inappropriate. Until axis homology can be identified, it seems prudent to use phylogenetically neutral terms to describe body axes.

## VI. CONCLUSIONS

(1) There is significant potential to improve our knowledge of development in Ediacaran macro-organisms, but the synthesis of existing data allows us to refute several previously proposed phylogenetic affinities for key Ediacaran taxa. Analysis of development in rangeomorphs and dickinsoniomorphs reveals congruence with aspects of metazoan development.

(2) We conclude that developmental data alone allow us to identify *Dickinsonia*, *Andiva*, *Yorgia* and the rangeomorphs as early metazoans.

(3) Morphogenesis offers promise for disentangling Ediacaran phylogenetic relationships and the evolution of development. Although the study of ontogeny is the study of change over time, by adopting a largely morphological approach when considering Ediacaran organisms, the 'change' has been largely overlooked. Future study of populations of organisms will allow better quantification of this change, as well as the production of growth models, both of which will ultimately increase the precision of phylogenetic resolution of Ediacaran organisms.

(4) The recognition of some the most enigmatic members of Ediacaran fossil assemblages as probable metazoans offers support to recent suggestions of considerable developmental complexity in early-branching metazoans (e.g. Ferrier, 2015), and lends credence to the idea that the early metazoan tree cannot be rationalised in terms of gradually increasing complexity, but may have followed a much more cryptic path.

## VII. ACKNOWLEDGEMENTS

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# ANATOMY OF THE EDIACARAN RANGEOMORPH *CHARNIA MASONI*

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**Abstract:** The Ediacaran macrofossil *Charnia masoni* Ford is perhaps the most iconic member of the Rangeomorpha: a group of seemingly sessile, frondose organisms that dominates late Ediacaran benthic, deep-marine fossil assemblages. Despite *C. masoni* exhibiting broad palaeogeographical and stratigraphical ranges, there have been few morphological studies that consider the variation observed among populations of specimens derived from multiple global localities. We present an analysis of *C. masoni* that evaluates specimens from the UK, Canada and Russia, representing the largest morphological study of this taxon to date. We describe substantial morphological variation within *C. masoni* and present a new morphological model for this species that has significant implications both for interpretation of rangeomorph architecture, and potentially for existing taxonomic schemes. Previous reconstructions of *Charnia* include assumptions regarding the presence of structures seen in other rangeomorphs (e.g. an internal stalk) and of homogeneity in higher order branch morphology; observations that are not borne out by our investigations. We describe variation in the morphology of third and fourth order branches, as well as variation in gross structure near the base of the frond. The diagnosis of *Charnia masoni* is emended to take account of these new features. These findings highlight the need for large-scale analyses of rangeomorph morphology in order to better understand the biology of this long-enigmatic group.

**Key words:** Ediacaran, rangeomorph, morphology, intraspecific variation, taxonomy.

THE emergence of animals is among the most formative evolutionary events in Earth history, yet our understanding of early animal evolution remains poorly constrained. Molecular estimates place the origin of Metazoa somewhere between 700 and 800 million years ago (dos Reis *et al.* 2015) but few body fossils of undisputed animal affinity are known from strata older than latest Neoproterozoic (e.g. Cunningham *et al.* 2017). Some of the best candidates for pre-Cambrian animals are members of the Ediacaran macrobiota: a disparate group of largely soft-bodied macroscopic organisms that lived in marine environments during the final *c.* 30 million years of the Ediacaran Period (Grazhdankin 2014; Budd & Jensen 2017). Despite the potential significance of these fossils for understanding early animal evolution, only a small number of Ediacaran macrofossil taxa have been morphologically well-characterized following study of large populations of individuals (e.g. Vickers-Rich *et al.* 2013; Evans *et al.* 2017; Hoekzema *et al.* 2017; Kenchington & Wilby 2017). Typical preservation of the Ediacaran macrobiota (as cast and mould impressions) means that there is uncertainty as to how much of their anatomy is captured, with internal features being particularly rare (though see Dzik & Ivantsov 2002; Narbonne *et al.* 2009; Vickers-Rich *et al.* 2013). Consequently, most previous suggestions of metazoan affinity for Ediacaran macrofossil taxa are equivocal and based on palaeoecological or developmental evidence in addition to the limited amount of direct morphological information currently available.

The earliest known palaeocommunities of the Ediacaran macrobiota date to *c.* 571–560 Ma (Noble *et al.* 2015; Pu *et al.* 2016) and are found among sedimentary rocks deposited in deep marine palaeoenvironments (e.g. Wilby *et al.* 2011; Liu *et al.* 2012). They are dominated


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by organisms with a frondose body plan that could reach up to two metres in length (Narbonne & Gehling 2003; Liu *et al.* 2015). Some of these fronds exhibit self-similar (sometimes considered 'fractal') branching and have been assigned to the morphogroup Rangeomorpha (Pflug 1972; Jenkins 1985; Narbonne 2004; Erwin *et al.* 2011), which may comprise a clade (Dececchi *et al.* 2017). The constructional architecture of rangeomorphs has proven difficult to reconcile with the body plans of extant taxa, resulting in multiple competing hypotheses, including both metazoan and non-metazoan affinities, for members of the group. These interpretations have included algae (Ford 1958), fungi (Peterson *et al.* 2003), lichens (Retallack 1994), total-group metazoan (Budd & Jensen 2017) and pennatulacean cnidarians (Glaessner 1959). Recent reassessment of developmental data derived from rangeomorphs concluded that most of these interpretations are not compatible with morphogenetic evidence and that rangeomorphs are likely to fall within the total group Metazoa (Dunn *et al.* 2018*a*).

Recent field and museum visits in Newfoundland (Canada), Charnwood Forest (UK) and the White Sea (Russia) have unearthed new material that includes rangeomorph specimens of markedly different sizes within individual species. Such specimens are interpreted as different developmental stages of the organisms (Liu *et al.* 2012; Wilby *et al.* 2015; Dunn *et al.* 2018*a*) that provide new opportunities to obtain insight into both rangeomorph anatomy and morphogenesis. The prominent rangeomorph taxon *Charnia masoni* (Ford 1958; Fig. 1A) has a long history of research, broad spatial and stratigraphical distributions and both shallow- and deep-marine environmental tolerance (Grazhdankin *et al.* 2008; Gehling & Droser 2013; Liu *et al.* 2015). New populations of *C. masoni* offer excellent opportunities to test claims of animal ancestry in Ediacaran rangeomorphs.

We here present a reanalysis of the morphology of *Charnia masoni* and identify features that lead us to propose a new model for its anatomy. This model has significant implications for our understanding of rangeomorph intra-specific variation, and consequently for rangeomorph taxonomic schemes. The following redescription is undertaken in the expectation that a detailed understanding of anatomy must necessarily precede understanding of an organism's place in phylogeny and, consequently, its evolutionary significance.

## PREVIOUS WORK

*Charnia masoni* is a uniterminal rangeomorph (see Dunn *et al.* 2018*a*), which is known to range in length from *c.* 1 to 66 cm (Fig. 1; Boynton & Ford 1995; Hofmann *et al.* 2008; Liu *et al.* 2012). It comprises a holdfast, stem and tapering ovate to parallel-sided frond (Laflamme *et al.* 2007) consisting of two rows of first order branches (Fig. 1A; terminology follows Brasier *et al.* 2012). First order branches are longest in the middle of the frond and shortest at the distal tip (Ford 1958). *C. masoni* is considered to belong to the Charniida (Pflug 1970; Glaessner 1979); a sub-group of Rangeomorpha comprising those taxa with single-sided (rotated; Brasier *et al.* 2012) first order branches (Narbonne *et al.* 2009). The angle of repose of *Charnia* first order branches varies amongst specimens (both within and between bedding planes) but the form of the organism remains constrained (Dunn *et al.* 2018*a*). First order branches meet in an alternating arrangement at the midline to form a zigzag apico-basal axis, with no visible stalk (Ford 1958; Grazhdankin 2004*a*) and, as such, the growth axis has been considered concealed (Brasier *et al.* 2012). This branch alternation confers glide reflection symmetry (an offset form of bilateral symmetry; e.g. Brasier *et al.* 2012) on the frond. Rarely, groups of first order branches may dislocate from their neighbours (Wilby *et al.* 2015, figs 5–10) but more commonly they present as a tightly stacked arrangement.

First order branches have been described to comprise up to 25 second order branches (Wilby *et al.* 2015), the shape of which may vary from rectangular to sigmoidal along an individual first order branch (Laflamme *et al.* 2007). Second order branches themselves comprise smaller, third (Jenkins 1985) and fourth order branches (Brasier & Antcliffe 2009), with each successive branch order oriented broadly perpendicular to the previous one. The branching in *Charnia masoni* has been described as undisplayed and furled at all orders (*sensu* Brasier *et al.* 2012), with the number of first order branches generally increasing with specimen size (e.g. Antcliffe & Brasier 2008). These observations have led researchers to conclude that *C. masoni* differentiated new first order branches during its life (*sensu* Dunn *et al.* 2018*a*) and that these branches subsequently inflated as the organism grew further (Antcliffe & Brasier 2007, 2008; Wilby *et al.* 2015). New branches have typically been interpreted to differentiate from the apex of the organism (Antcliffe & Brasier 2007), where the smallest first order branches are located, but an additional basal growth zone has been proposed following identification of stems of markedly different relative lengths in some specimens (Dunn *et al.* 2018*a*, figs 1A–B, 2E). Whether all four orders of branch division are visible at all observed stages of ontogeny, or whether they emerge during development in a hierarchical fashion (as suggested by Flude & Narbonne 2008), has not yet been resolved.

Although the gross morphology of *Charnia masoni* has been relatively well-characterized, discrepancies exist in the detail to which its component parts have been studied. The morphology of first order branches has been well analysed (e.g. Laflamme *et al.* 2007; Wilby *et al.* 2015),

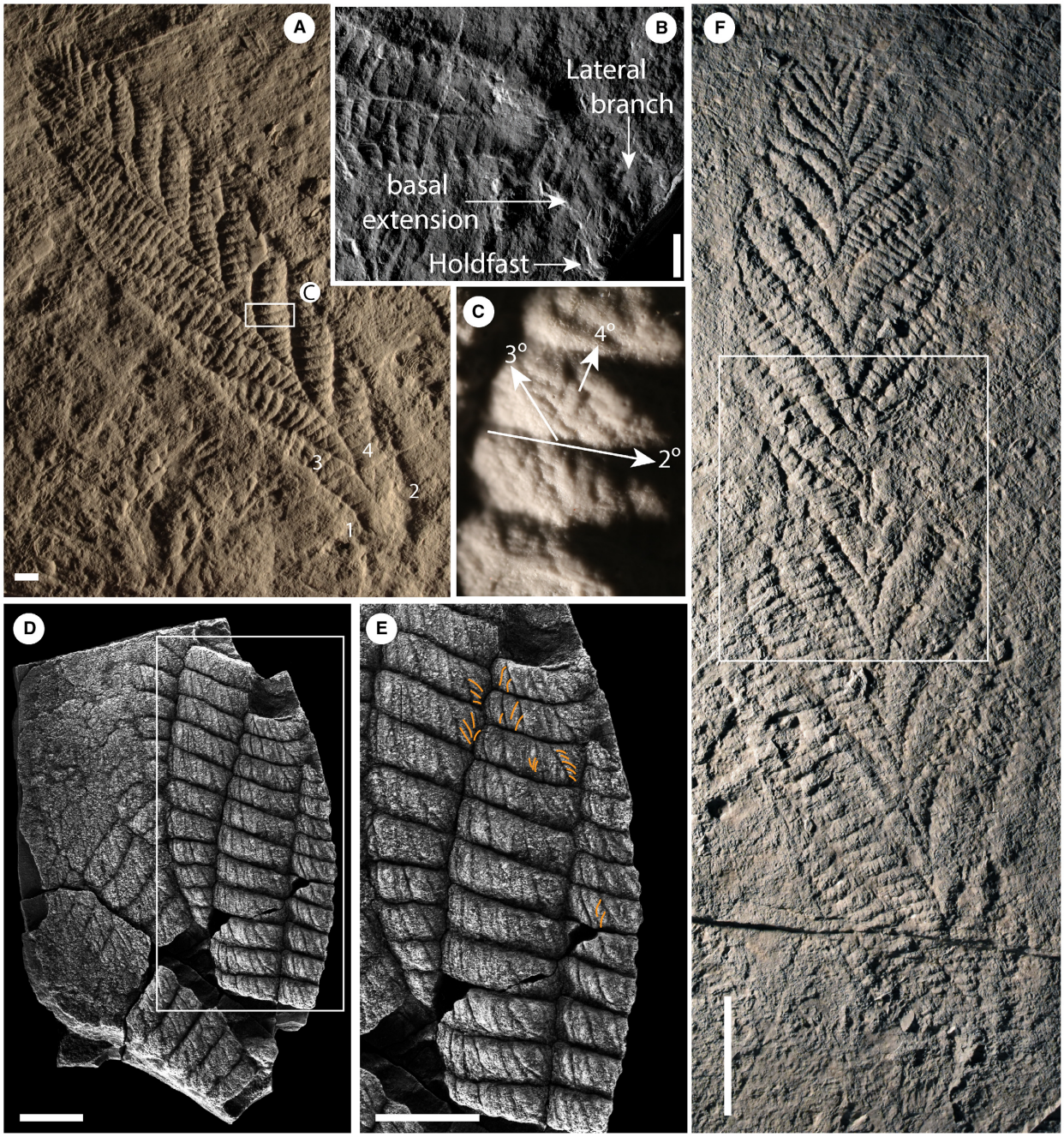



**FIG. 1.** A–C, *Charnia masoni* Holotype (LEIUG 2328) from Bed B (Wilby *et al.* 2011), North Quarry, Charnwood Forest, UK: A, latex mould of the complete specimen; lateral branches (the basal-most branch pair) are labelled 1 and 2, branches comprising the basal extension (the next most basal branch pair) are labelled 3 and 4; see Dunn *et al.* (2018*b*) for a reflectance transformation image of holotype specimen; B, cast of the basal region of the holotype, showing the holdfast, basal extension and lateral branches; C, displayed branch architecture in third and fourth order branches (2°, second order branch; 3°, third order branch; 4°, fourth order branch); holotype mould. D–E, partial *Charnia masoni* specimen from the White Sea (PIN 3993-7018): E, high order rangeomorph branching, examples of rotated or displayed furled fourth order branches are highlighted in orange; F, latex mould of a *Charnia* specimen interpreted as being twisted by Wilby *et al.* (2015) (BGS GSM 105873); the white box highlights the area of inferred twisting. Scale bars represent: 10 mm (A–E); 5 cm (F).

while third order and fourth order branches have been little discussed in the literature, presumably due to their small size and incomplete preservation within most specimens. There is therefore ample scope for morphological analysis of these smallest branch divisions using well preserved specimens.

*Charnia masoni* is widely considered to have been identical on both faces/sides. However, Grazhdankin (2004*a*) suggested that this may not have been the case and that one face of *C. masoni* possessed characteristic furled and rotated rangeomorph branching architecture at multiple branch orders, while the other possessed first and second order branches only. Narbonne *et al.* (2009) described putative internal anatomy in one specimen termed '*Charnia* cf. *C. masoni*', identifying a possible central stalk with 'tube'-like support structures for the first order and second order branches. They also describe an outer 'distal rim' to the frond, which they considered was an internal feature that originally connected to the central stalk and the first order branch support structures (though see Grazhdankin & Seilacher 2005, who interpreted 'internal' structures as resulting from current winnowing, or Brasier *et al.* 2013, who reinterpreted both the distal-rim and the internal stalk as sedimentary features related to scouring).

The holdfast of the organism has received little discussion (though see Jenkins 1985; Grazhdankin 2014), possibly because much work has focused on the holotype specimen, in which the holdfast has historically been thought to be missing (though see Wilby *et al.* 2015 fig. 5-1). Where present, the holdfast is small and bulbous (Laflamme *et al.* 2007; Wilby *et al.* 2015), though it was described as elongate by Jenkins (1985) and recent work has also suggested that the holdfast of *Charnia masoni* may be more deeply buried than other rangeomorph holdfasts, thus only appearing to be smaller (Burzynski & Narbonne 2015). In a few specimens, a stem-like region (Dunn *et al.* 2018*a*), sometimes with second order subdivisions (Wilby *et al.* 2015), can be seen in *C. masoni* connecting the holdfast to the frond (the basal extension as defined here). This region is considered distinct from the true, naked stems of other rangeomorphs (Laflamme *et al.* 2012) and non-rangeomorph frondose Ediacaran taxa (e.g. Laflamme *et al.* 2004), which do not possess any second order subdivisions along their stems.

In summary, while *Charnia masoni* is one of the best studied rangeomorph taxa, there remain several crucial aspects of anatomy that are either contentious (e.g. internal anatomical structures), or insufficiently characterized. Some of these are features (e.g. branching architecture) that contribute significantly to taxonomic diagnosis in rangeomorphs (Laflamme & Narbonne 2008; Brasier *et al.* 2012). Any improvement to our knowledge of the anatomy of *Charnia* is therefore valuable.

## MATERIAL AND METHOD

A total of 47 well preserved *Charnia masoni* specimens from Bed B of North Quarry in the Bradgate Formation, Charnian Supergroup, UK (see Wilby *et al.* 2011), including the holotype (LEIUG 2328, Ford 1958), and 17 specimens from Bed LC6 of the Catalina Member of the Trepassey Formation, Newfoundland (see Liu 2016), were studied either in the field or from high resolution casts and moulds (figured specimens are housed at the British Geological Survey, Keyworth and the Sedgwick Museum, Cambridge, respectively). Specimens are preserved in low negative epirelief and occupied deep-water turbiditic depositional settings during life (Wood *et al.* 2003). Five additional partial specimens from the Verkhovka Formation, Valdai Group, White Sea region of Russia (Grazhdankin 2004*a*), were analysed from photographs, or at the Paleontological Institute (PIN) in Moscow. These Russian specimens are preserved in three dimensions in fine-grained sandstone interbeds alternating with mudstone and representing a storm-influenced middle shoreface depositional environment (Grazhdankin 2004*a*).

Specimens of *Charnia masoni* from Newfoundland were retrodeformed prior to study (a technique used to account for tectonic deformation of specimens; Wood *et al.* 2003) following the constant area method (Heywood 1933), while specimens from Charnwood Forest were not retrodeformed since all fronds on Bed B are aligned and are considered to have been subjected to the same magnitude of deformation (following Wilby *et al.* 2015). Specimens from the White Sea were not retrodeformed, as the strata are not considered to have undergone significant tectonic deformation (Stankovsky *et al.* 1990; Grazhdankin 2003, 2004*b*). Due to inherent deformational differences, we do not consider quantitative data derived from these various populations to be directly comparable. However, we do discuss general morphological variation across the different sample areas.

Interpretive illustrations of individual specimens were produced in Adobe Photoshop CC. Silicone moulds were made of specimens from Newfoundland in the field, under permits issued by the Government of Newfoundland and Labrador, under Regulation 67/11 of the Historic Resources Act.

*Institutional abbreviations.* BGS, British Geological Survey, Keyworth, UK; CAMSM, Sedgwick Museum, University of Cambridge, UK; LEIUG, Department of Geology, University of Leicester, UK; OUMNH, Oxford Museum of Natural History, Oxford, UK; PIN, Paleontological Institute, Russian Academy of Sciences, Moscow, Russia.

## RESULTS

### *Specimens from Charnwood*

The best-preserved and largest specimens of *Charnia masoni* exhibit four (resolvable) orders of branching

(Fig. 1), while the smallest specimens (*c.* 2 cm) lack the resolution required to determine the number of branch orders originally present. The smallest first order branches are located at the distal tip of individual fronds, which are typically ovate in shape and appear well constrained (i.e. lacking first order branches of aberrant length) in all specimens. One specimen appears to show an area of first order branch dislocation (*sensu* Wilby *et al.* 2015) with the angle of repose of first order branches being higher above the dislocated area (towards the distal tip; Fig. 1F). First order branches are constructed of rectangular second order branches, which are oriented laterally and basally and are themselves constructed of third and fourth order branches. Third order branches, which are oriented apically, can appear displayed and furled (Fig. 1C, terminology *sensu* Brasier *et al.* 2012), undivided, or rotated and furled.

Most first order branches appear to meet in an alternating arrangement in the centre of the organism, conferring a glide symmetrical arrangement. However, the two most proximal branches in individual specimens (closest to the holdfast) do not appear to conform to this pattern, instead connecting directly to the lateral margins of the holdfast (Figs 1A–B, 2). These two most proximal branches (observed to be present in eight specimens and absent from eight specimens, based on the position of their unique attachment point) are distinct from all other first order branches, with second order branches present along their entire length and third order branches sporadically preserved. We term this pair of first order branches the lateral branches. The next most-apical pair of first order branches (i.e. the second pair of first-order branches; Figs 1A–B, 3) may also appear morphologically distinct, in some cases extending between the most proximal first order branch pair (the lateral branches) to form an area previously termed the 'stem' or stem-like area (Dunn *et al.* 2018*a*). This area is variable among specimens; it can be present or absent within individuals from a single population (it is present in 9 specimens from Charnwood Forest, out of 19 where the base of the organism is preserved) and it may vary in length within the population (both in absolute and proportional terms; see Fig. 4, Table 1).

A stalk-like structure may be visible near the base of the frond in one specimen (Fig. 5A, B) and appears to connect directly to the holdfast (NB a stalk runs apico-basally through the frond, and the stem connects the holdfast to the frond, *sensu* Brasier *et al.* 2012). However, similar structures in other specimens appear to be the remains of first order branch boundaries where the branches have been effaced (Fig. 5C, D). Such structures should, therefore, be treated with caution. Where first order branches appear dislocated (Fig. 1F), there does not appear to be any suggestion of a central stalk structure.

A holdfast is not observed in the majority of *Charnia masoni* specimens from Charnwood Forest but where it is observed (16 specimens) it varies from circular to slightly elongate in shape and is generally small (relative to other rangeomorph holdfast structures; e.g. Wilby *et al.* 2011, fig. 2B–C). The possibility remains that it could be deeply buried and therefore not preserved in its entirety on the bedding plane (Burzynski & Narbonne 2015).

### *Specimens from Newfoundland*

*Charnia masoni* specimens from Newfoundland include small individuals measuring little over 1 cm in length (Liu *et al.* 2012) and possessing three resolvable orders of branching (Fig. 3). Larger specimens may display up to four resolvable orders of branching, with specimens appearing to fall into two distinct morphs that generally show little/no spatial overlap, but which can co-occur on individual beds. One morph possesses an ovate frond outline, and resembles specimens from Charnwood Forest (e.g. Fig. 6E). The other morph exhibits a slender and strongly parallel-sided frond (cf. Laflamme *et al.* 2007; Figs 7, 8). Both morphs have a constrained frond form, with the smallest first order branches present at the distal tip of the frond and the longest first order branches present in the middle, with first order branches meeting in the centre of the frond in an alternating arrangement. In the parallel-sided morph, which is present on at least five distinct surfaces, second order branches appear sigmoidal in shape, where their lateral margins are preserved. Third order branches may be undivided and furled, or rotated and furled (*sensu* Brasier *et al.* 2012; Fig. 8D). Taphonomic constraints prohibit us from drawing conclusions regarding the morphology of the smallest branching orders in the Charnwood-type morph.

In certain specimens of the parallel-sided morph from two individual bedding planes in Newfoundland (LC6 and Site 40 of Hofmann et al. 2008), the frond is connected to the holdfast via a long connecting region that is narrower than the frond (Figs 7–8). On both beds, *Charnia masoni* specimens with this connecting region are considerably more abundant than specimens without (no specimens without the connecting region are documented at Site 40, while only two are documented on LC6, in contrast to *c.* 20 specimens that possess a connecting region). This area is commonly preserved in positive epirelief, in contrast to the negative epirelief preservation of the frond branches (Fig. 7D). It may display first and second order branching at least part way along its length (Figs 7A–C, E; 8), with a bias towards preservation of only one row of first order branches (e.g. Fig. 7B, C). Within this connecting region, effaced first and second

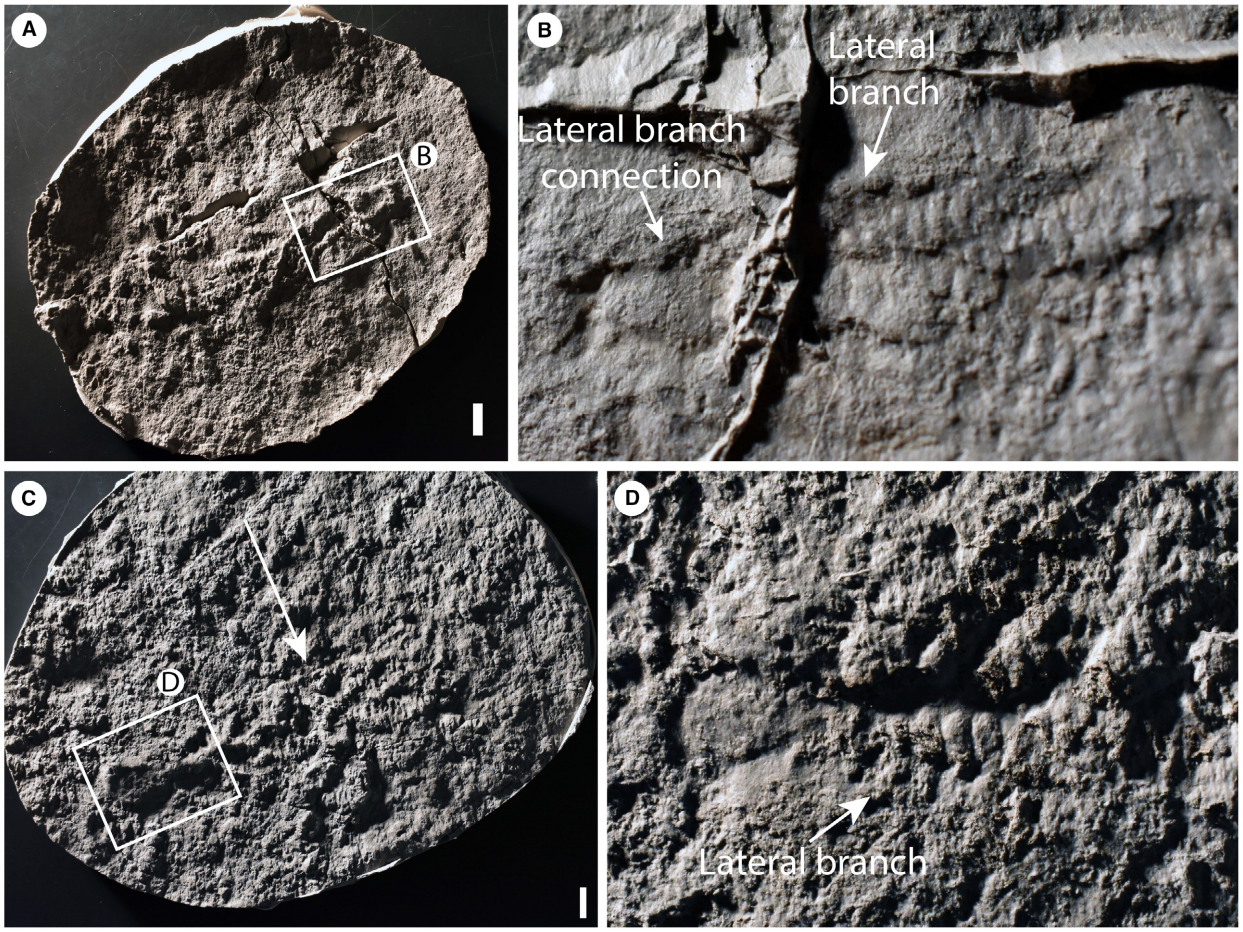



**FIG. 2.** *Charnia masoni* specimens from Charnwood Forest, UK. A–B, cast of BGS GSM 105993; the arrows in B highlight the basal-most branch as it connects directly to the lateral margin of the holdfast. C–D, cast of BGS GSM 105972; the specimen is arrowed in C; in D, the arrow points to the basal-most branch, which connects directly to the lateral margin of the holdfast. Scale bars represent 10 mm. Colour online.

order branching is commonly visible (e.g. Figs 7, 8). The length of the connecting region located proximally to the basal-most expression of distinct first or second order branching is variable within populations (Fig. 9; Table 2) and is not tightly correlated to specimen size. A holdfast is commonly preserved in specimens from Newfoundland, and can exhibit circular to slightly elongate morphologies (Figs 7, 8).

*Specimens from Russia*

All examined specimens from the White Sea are incomplete and so no comments about gross form can be made. Four orders of branching were noted in well preserved areas (Fig. 10D, E), and first order branch form appears constrained. First order branches meet along the midline in an alternating fashion, conferring glide symmetry upon the frond. The exposed area in Figure 1D–E (Grazhdankin 2004*a*, fig. 2B) highlights the tight packing of first order branches. We find no evidence for a central stalk in this exposed area, or in any of the Russian specimens. As with specimens from Newfoundland, second order branches may be rectangular or sigmoidal (furled or displayed; Fig. 10D). Where second order branches are disarticulated (e.g. Fig. 10D), the boundary between these branches appears clean. Third order branches may appear either furled and undivided (Fig. 10A, B), rotated and furled, or displayed and furled (Figs 1D–E, 10E). As with specimens from Newfoundland, the basal margins of third order branches (across one second order branch) are more evenly spaced than the apical margins, which appear to be oriented medially in many cases (e.g. Fig. 10A, B), suggesting that the third order branches attach to a support structure located basally in each second order branch.

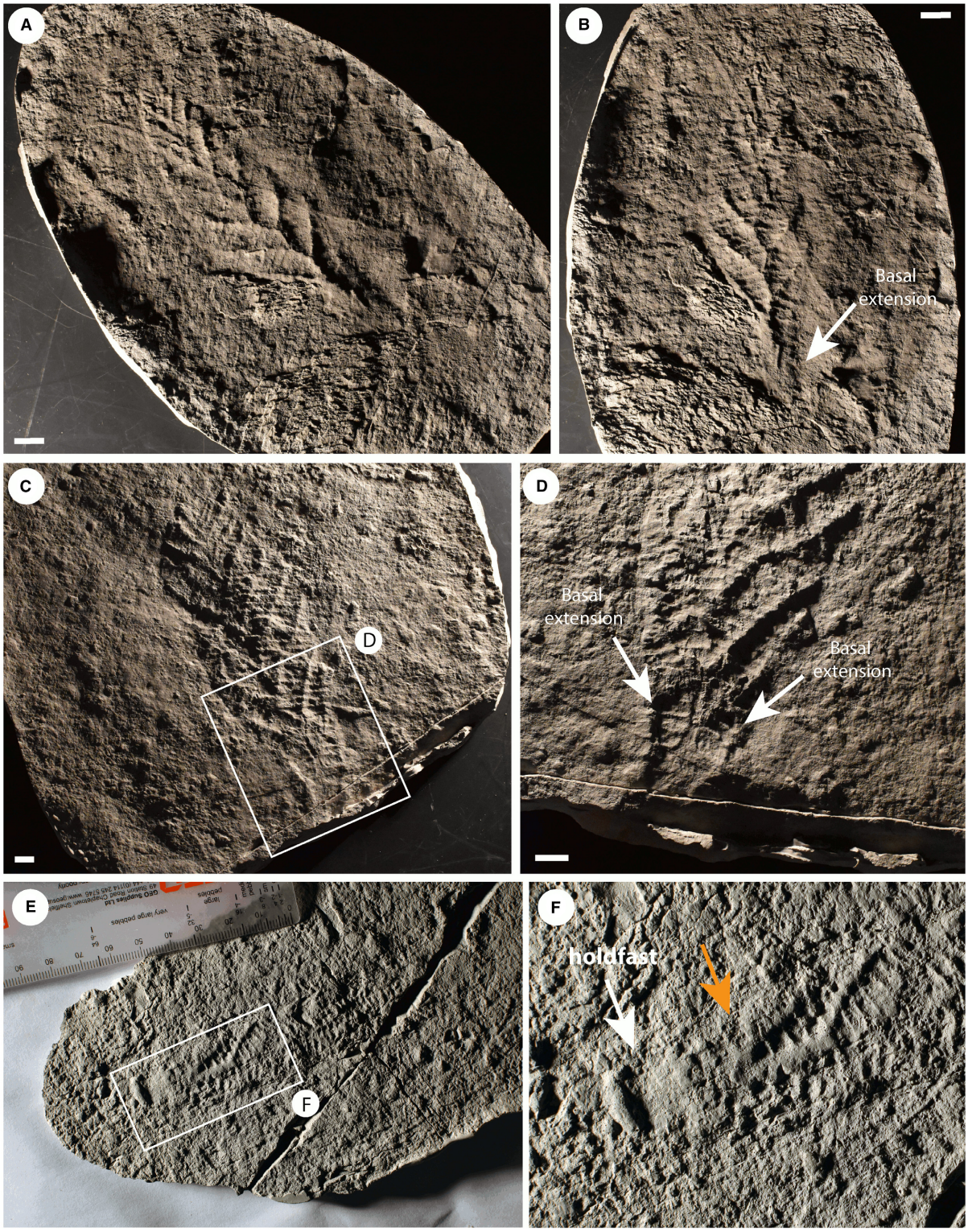



**FIG. 3.** *Charnia masoni* specimens from Charnwood Forest, UK. A–B, cast of BGS GSM 106078, showing the basal extension. C–D, cast of BGS GSM 105997, showing the basal extension in D; this specimen does not preserve a holdfast. E–F, cast of BGS GSM 105966, which does not show a basal extension, but rather the first order branches connect to the holdfast without any expansion near the base of the branch; the holdfast and lowermost branches are arrowed in F (left and right arrows respectively). Scale bars represent 10 mm. Colour online.

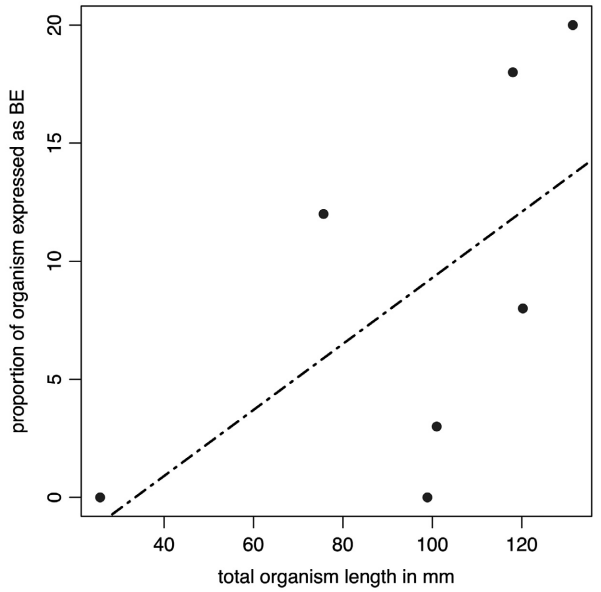



**FIG. 4.** Data from Table 1 plotted in graphical form. The black dashed line represents the best fitting (linear) model (AICc = 59.12972), but this is non-significant ($p$ = 0.1483).

**TABLE 1.** Measurements of total specimen length, the length of the basal extension and the relative proportion of the specimen this area comprises.

| Specimen | Total length (mm) | Basal extension (mm) | Length of the basal extension as a proportion of total organism length (%) |
|---|---|---|---|
| GSM 105978 | 118 | 20.84 | 18 |
| GSM 106040 | >111.31 | 11.61 | N/A |
| GSM 105966 | 98.89 | 0 | 0 |
| Holotype | >220.09 | 26.61 | N/A |
| GSM 106078 | 131.41 | 26.26 | 20 |
| GSM 105989 | 75.66 | 8.86 | 12 |
| GSM 105979 | 100.97 | 3.40 | 3 |
| GSM 105997 | >173.90 | 26.51 | N/A |
| GSM 105972 | 120.24 | 9.64 | 8 |
| GSM 106084 | 25.7 | 0 | 0 |

'N/A' represents cases where the total length data are not precise, and therefore proportions cannot be accurately determined. Specimens from Bed B, Charnwood Forest, UK; housed at BGS.

## DISCUSSION

Integration of the information above allows construction of a new morphological model that better reflects the anatomy of *Charnia masoni* (Fig. 11). In the following section, we first discuss the frond and then move basally down the organism to the holdfast.

First order branches in *Charnia masoni* were already known (albeit rarely) to dislocate from each other (Wilby *et al.* 2015), suggesting the presence of only a weak connection between adjacent branches or, alternatively, a stacked arrangement of non-conjoined branches (bound together only at the central axis, or alternatively attached to an axis independent of each other). Evidence indicating that the basal margin of one first order branch could overlie the apical margin of the previous first order branch (Grazhdankin 2004*a*, fig. 2D; Laflamme *et al.* 2007) perhaps supports the latter hypothesis. We do not find evidence for a marginal rim (*sensu* Narbonne *et al.* 2009), or any other connective structure inferred to surround first order branches. *Charnia masoni* possesses three further orders of branch subdivision (totalling four orders of branching). It is not currently possible to determine whether the observation that only three branching orders are visible in the smallest, presumed youngest, specimens results from ontogenetic or taphonomic processes.

First order branches are sigmoidal in shape and are constructed of second order branches that are rectangular to sigmoidal. Variation in second order branch morphology is the result of the degree of physical rotation each branch has undergone, with fully exposed branches appearing sigmoidal (e.g. Fig. 6; see also Laflamme *et al.* 2007), whereas rectangular second order branches appear to have been furled. Second order branches probably possessed their own boundary walls and so it is unlikely that they were joined to each other in life along their entire medial-distal axis; they were connected only at their medial margin. We therefore term this medial margin the first-order branch axis (Fig. 11C).

We see no evidence to suggest that first or second order branches in *Charnia masoni* could exhibit a displayed rangeomorph branching architecture in any examined specimens, consistent with previous suggestions of single-sided 'Charniid' branching at these branch orders (Narbonne *et al.* 2009; see also thin section data in Grazhdankin 2004*a*, fig. 2d.)

While the majority of third order branches appear to conform to the typical furled, rotated or undivided, rotated pattern that defines the genus (e.g. Brasier *et al.* 2012; Wilby *et al.* 2015) individual branches at these higher orders may be furled and displayed, while some are unfurled and displayed (Fig. 1C). Given the apical orientation of displayed third order branches in specimens from Charnwood Forest, as well as the apical margins of third order branches in specimens from Russia being oriented medially (thus suggesting they were not bound at this margin), third order branches are interpreted as branching apically from their host second order branch along a second order branch axis (Fig. 11C). Third order branches also exhibit moderate inflation

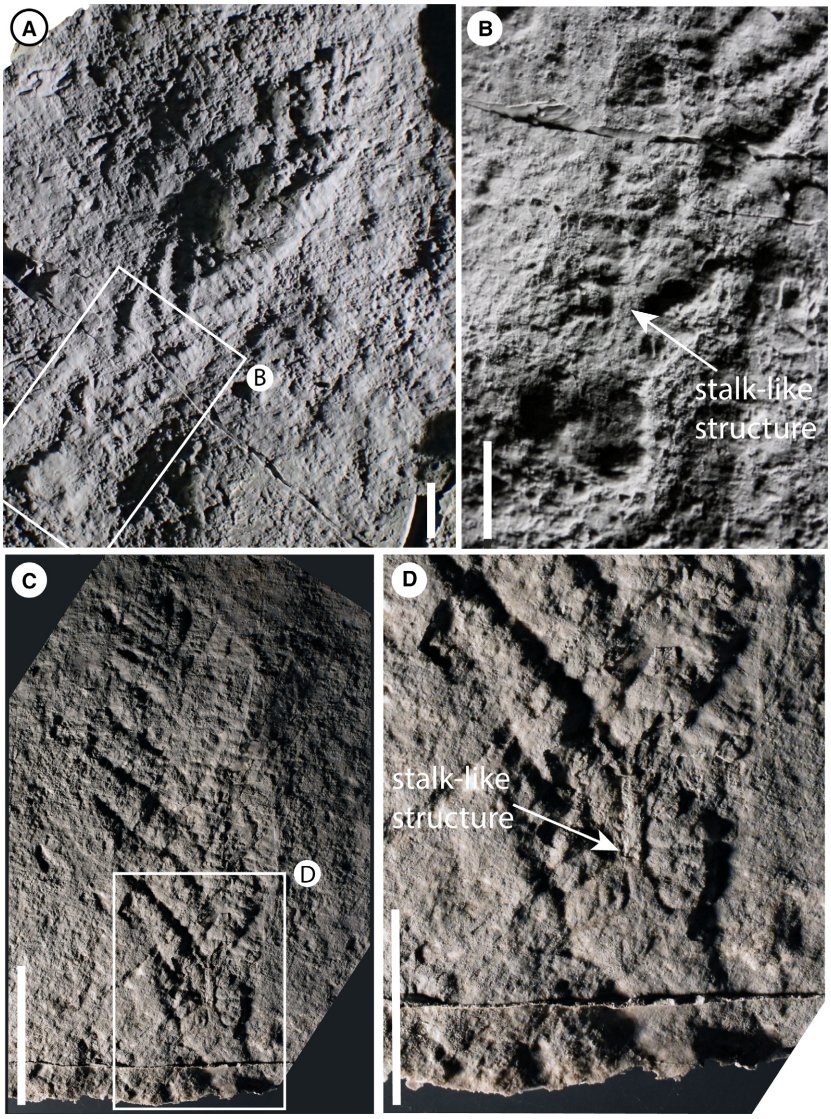



**FIG. 5.** A–B, *Charnia masoni* specimen cast (BGS GSM 105989), Charnwood Forest, UK; B, base of the specimen in A, showing first order branches connecting to a stalk-like structure (arrowed). C–D, mould of specimen BGS GSM 105997, showing what ostensibly appears to be a stalk-like structure; D, the stalk-like region, which appears to represent the effaced remnants of adjacent first order branches. Scale bars represent 10 mm. Colour online.

(*sensu* Brasier *et al.* 2012). Given the rotational variation we observe in fourth order branching, we consider it unlikely that third order branches were conjoined.

Fourth order branches have never been observed to show further hierarchical subdivision. We acknowledge that taphonomic constraints may preclude visualization of further branch orders but note that space constraints do not appear to limit the number of orders visible (e.g. Fig. 10E). Fourth order branches typically appear furled and may exhibit moderate (Fig. 1C) or medial (Fig. 10E) inflation. This is unlike the apparently conserved proximal inflation inferred for first order branches but similar to the moderate–medial inflation inferred for second order branches (Brasier *et al.* 2012).

These observations help to resolve the long-standing question regarding whether rotated (*sensu* Brasier *et al.* 2012) or 'charniid' branches (*sensu* Narbonne *et al.* 2009) have one or two rows. These specimens (from Charnwood, UK and the White Sea, Russia) demonstrate that rotated branches could be two-sided at higher branch orders, with one side rotated out of the plane of preservation (Fig. 11C). The potential for (at least third order) rotated branches to appear displayed (Fig. 10D, E), and furled branches to appear unfurled (Fig. 1C), suggests branching characters at higher (third and fourth) orders are not taxonomically conserved (see Kenchington & Wilby 2017). The rotation of these branches supports the notion that at least fourth order branches, and perhaps third order branches in *Charnia masoni*, were not conjoined, but free to move and rotate in the axial plane (cf. Wilby *et al.* 2015).

Branching architecture has significant bearing on the debate surrounding whether *Charnia masoni* had distinct front–back differentiation (see also Grazhdankin 2004*a*). We have been unable to corroborate the identification of two different faces to *C. masoni* in the *c.* 70 specimens

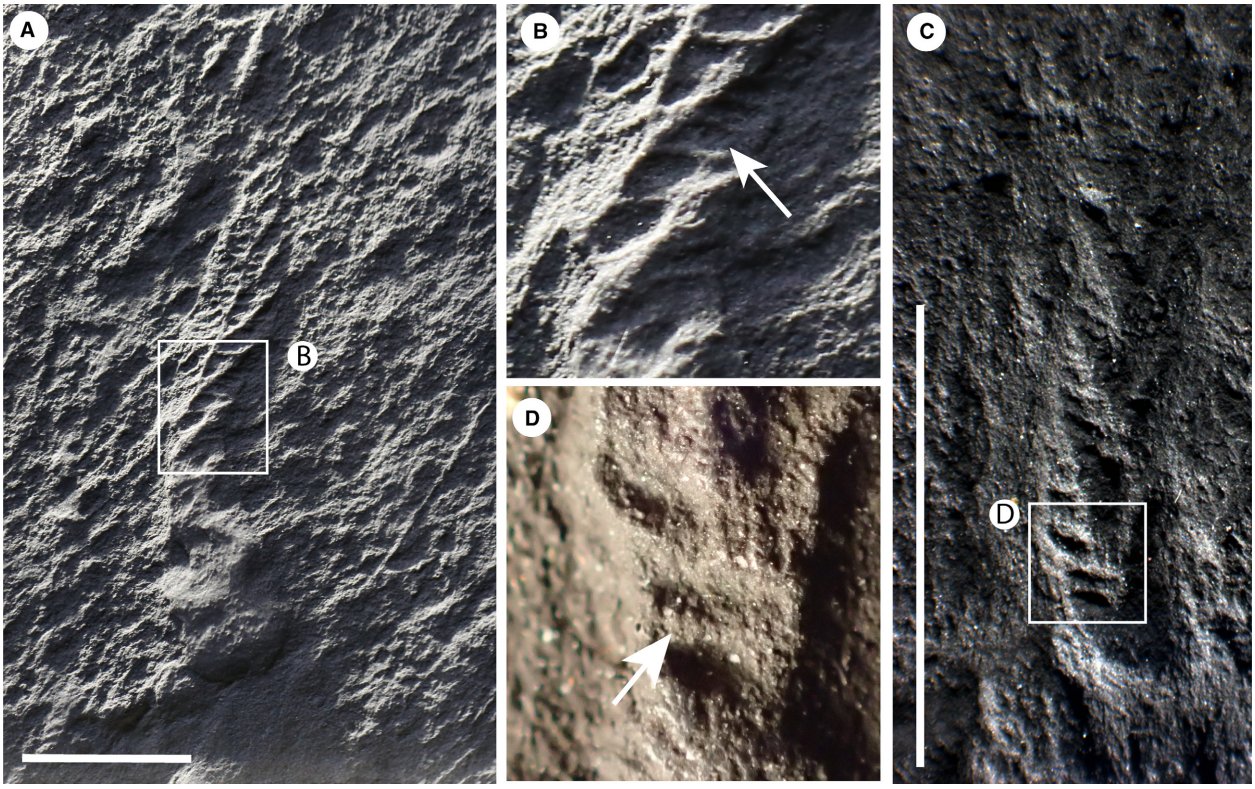
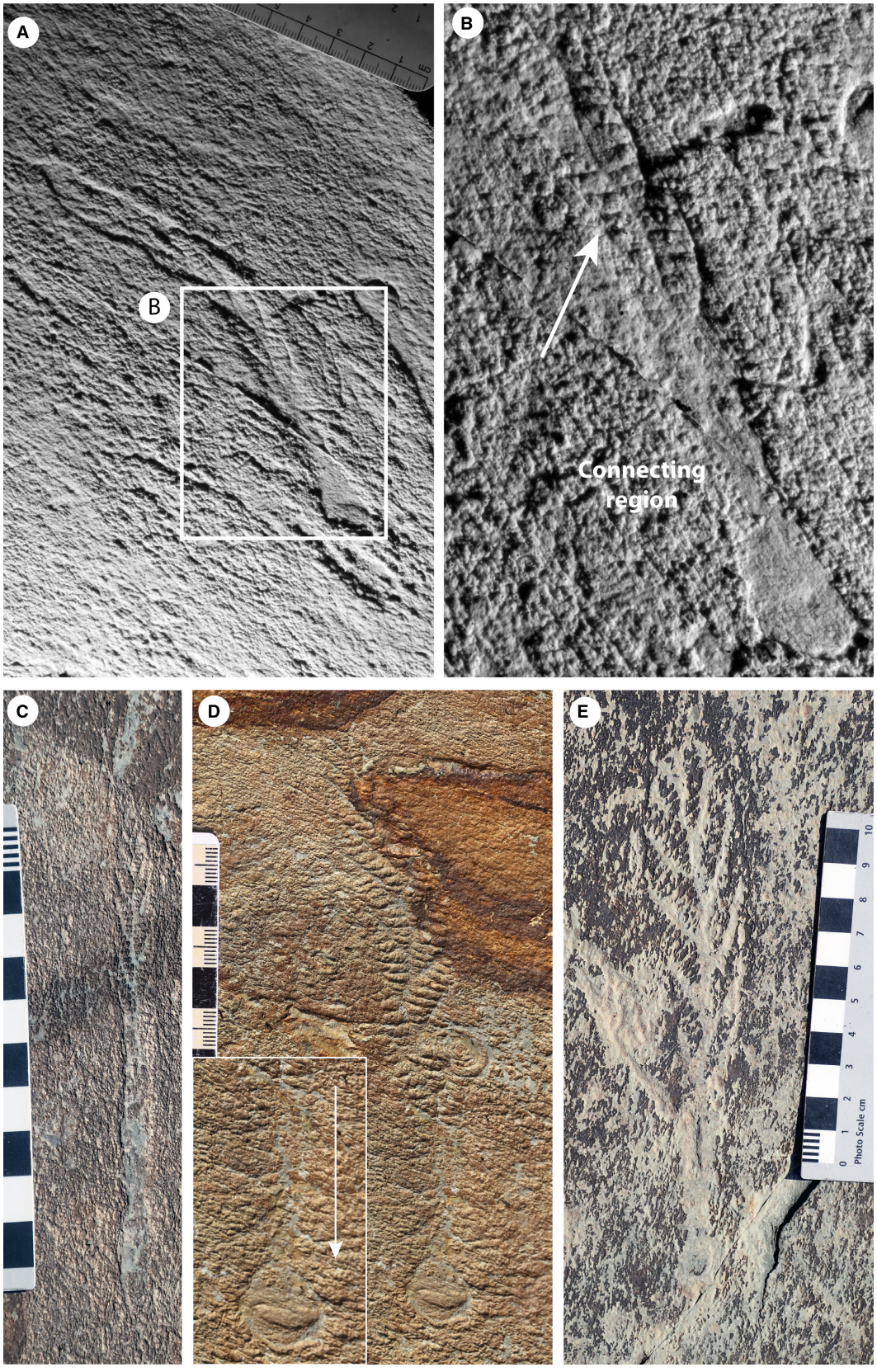



**FIG. 6.** A, *Charnia masoni* (cast) from the MUN surface, Newfoundland, Canada (Liu *et al.* 2016) (CAMSM X.50297.9) showing third order branching, highlighted in B. C–D, the smallest described specimen of *C. masoni* (OUMNH ÁT.429/p) from Pigeon Cove, Newfoundland, Canada (Liu *et al.* 2012) with third order branching highlighted in D. Scale bars represent 10 mm. Colour online.

directly studied here, and therefore infer that both sides of the organism probably possessed the same morphology (see also a Charnwood specimen inferred to be twisted (*sensu* Wilby *et al.* 2015) but displaying the same morphology above and below the twist: Fig. 1F). The apparent absence of third and fourth order branching in some specimens from the White Sea (Grazhdankin 2004*a*, fig. 2A) may then represent a taphonomic artefact. The considerable morphological variation in third and fourth order branches (as opposed to first and second order) may suggest that these finer orders of branching played a greater role in nutrient acquisition, as they were free to rotate around their axis. However, this greater flexibility could also simply be a function of their small size and not have been of functional significance. The lack of evidence for rangiid style branching in the first and second order branches may further suggest that *C. masoni* is not self-similar at every branch order (e.g. Narbonne 2004), although additional evidence is required to confirm or refute this. If this suggestion is borne out, this would undermine the current definition of Rangeomorpha, which requires orders of branching that are identical to 'at least three orders' (Erwin *et al.* 2011).

The lateral branches (Fig. 2) are morphologically distinct in terms of their unique attachment point to the holdfast, perhaps indicating a greater level of axial complexity to *Charnia masoni* than has previously been inferred (e.g. Hoyal Cuthill & Conway Morris 2014, though see Dunn *et al.* 2018*a*). The next most proximal pair of first order branches may also be morphologically distinct, in some cases extending between the two most proximal first order branches to form an area previously termed the 'stem' (e.g. Dunn *et al.* 2018*a*; Fig. 1B).

**FIG. 7.** Specimens of *Charnia masoni* from locality LC6, Bonavista Peninsula, Newfoundland, Canada. A–B, silicone mould of a slender (parallel-sided) specimen (CAMSM X. 50297.10) with what we term the 'connecting region', showing sigmoidal first order branching extending much of the way down the specimen, arrowed in B. C, parallel-sided specimen with a connecting region preserved in positive epirelief (cast of CAMSM X.50297.2). D, specimen with a basal extension in the connecting region (cast of CAMSM X.50297.1); arrow in the inset shows the branch connections to the holdfast. E, Charnwood-like specimen with first order branches showing 'connecting region' typical of parallel-sided specimens from this surface. Images are retrodeformed, except specimen in C due to lack of available holdfast structures. Main scales in cm. Colour online.


A
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B
Connecting region
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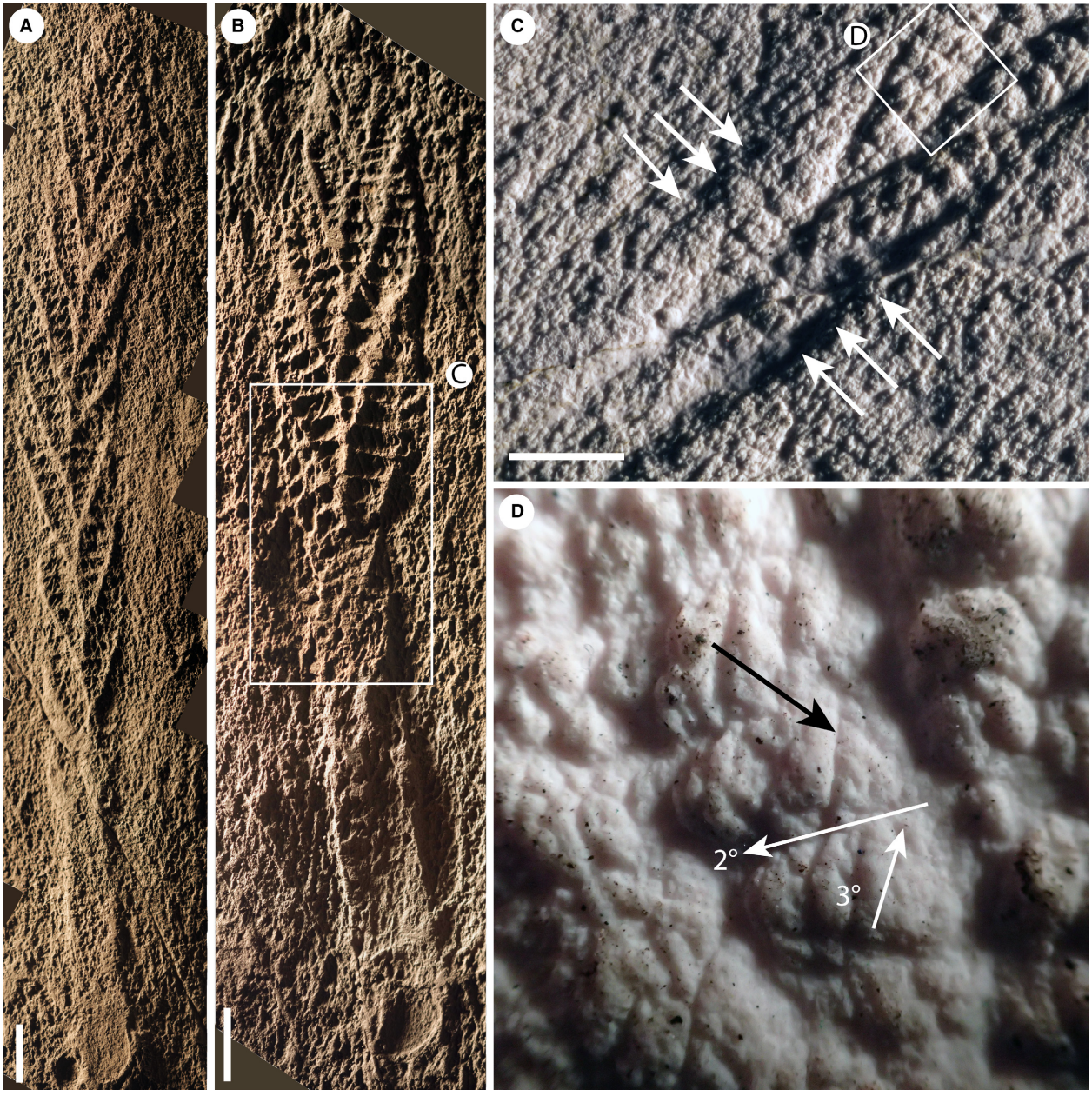



**FIG. 8.** Casts of specimens of *Charnia masoni* from Newfoundland, bed LC6. A, CAMSM X.50297.5. B, CAMSM X.50297.4. C, the basal area of the specimen in B, with second order branches visible (arrowed) on adjacent first order branches running down into the connecting region. D, rotated and furled third order branches, arrowed (black), from the specimen in B (orientation of second and third order branches indicated by white arrows). Images were retrodeformed using the constant area method. Scale bars represent 10 mm. Colour online.

However, because this area, where present, comprises two individual first order branches rather than a central fused region, we term this area the 'basal extension' (Figs 1A–B, 11). The basal extension displays some similarity to the proximal section of the subdivided 'axial stalk' (a stem as defined by Brasier *et al.* 2012) described in *Rangea schneiderhoehni* (Vickers-Rich *et al.* 2013). However, in *R. schneiderhoehni* this area is considered a single structure (i.e. not constructed of abutting first order branches). The basal extension is also distinct from the 'naked' stems of other rangeomorphs (e.g. Laflamme *et al.* 2012).

The parallel-sided morph of *Charnia masoni* from Newfoundland possesses a connecting region (Figs 7–8),

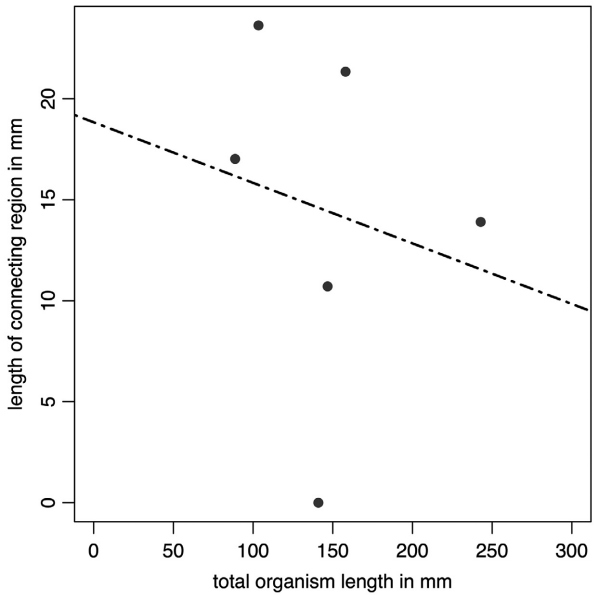



**FIG. 9.** Data from Table 2 plotted in graphical form. The black dashed line represents the best fitting (linear) model (AICc = 59.39492), but this is non-significant ($p$ = 0.7174).

**TABLE 2.** Comparison of specimen total length and connecting region length in specimens from locality LC6, Newfoundland, Canada.

| Specimen | Total length (mm) | Connecting region (mm) | Length of connecting region as a proportion of total organism length (%) |
|---|---|---|---|
| X. 50297.11 | 88.78 | 15.11 | 17.02 |
| X. 50297.7 | 103.4 | 23.40 | 23.63 |
| X. 50297.1 | 140.96 | 0 | N/A |
| X. 50297.10 | 146.76 | 15.72 | 10.71 |
| X. 50297.4 | 242.81 | 33.75 | 13.9 |
| X. 50297.5 | 158.01 | 33.72 | 21.34 |

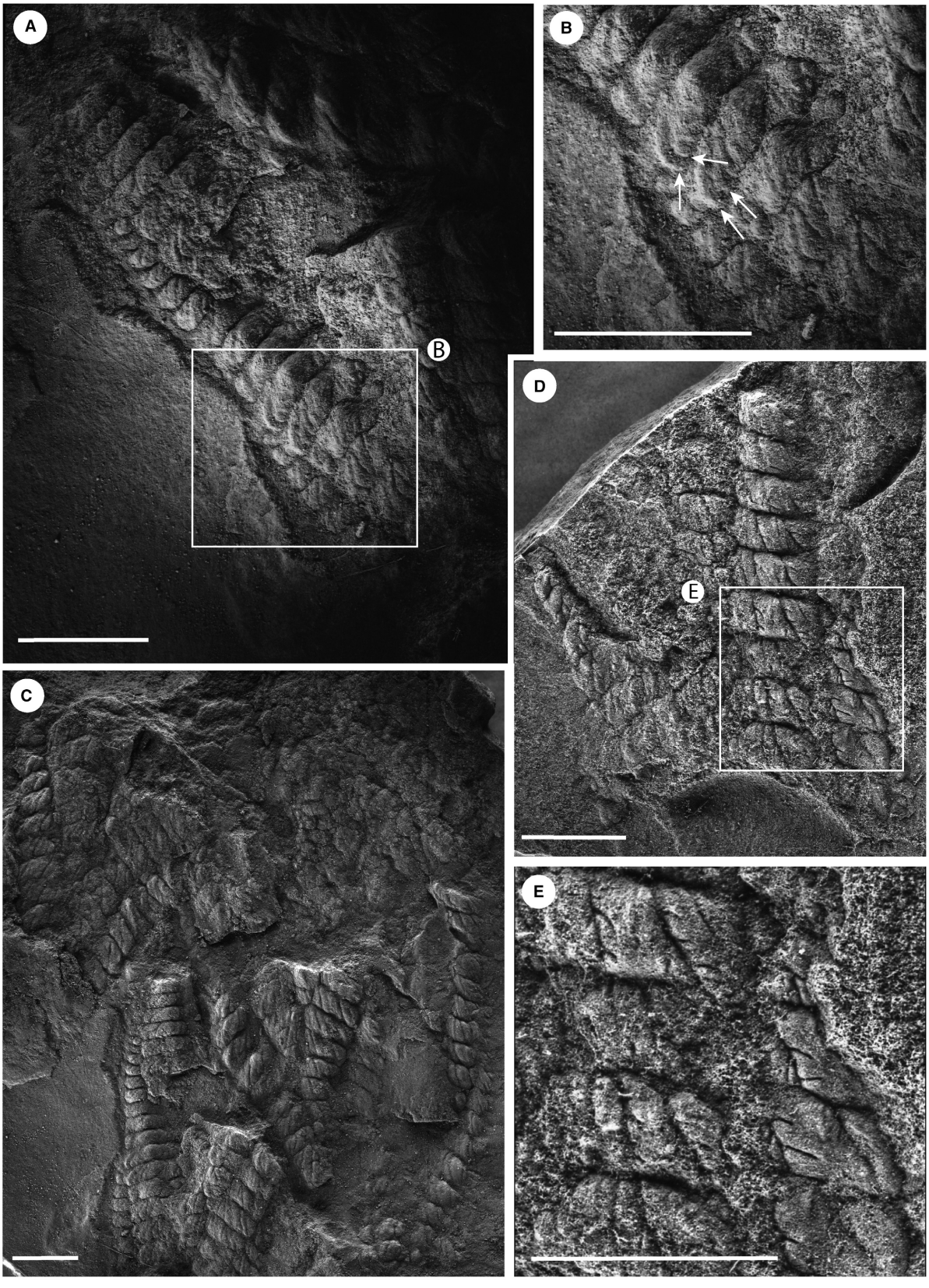
Only specimens where the base of the organism is well preserved were included in our analysis. 'N/A' represents cases where the total length data are imprecise, and therefore cannot be used to accurately determine proportions. Images were retrodeformed prior to measurement using the constant area method. Specimens housed at CAMSM.

although exact structural reconstruction of this region is hampered by variable quality of preservation near the base of the frond (resulting in often gradational boundaries between the branched area and 'naked' connecting region). This gradational zone, with what appear to be first order branches continuing down the 'connecting region' in many specimens (e.g. Figs 7A–B, 8A), suggests that this area does not represent a sheath structure (Narbonne *et al.* 2009; though see Brasier *et al.* 2013). This structure could alternatively be interpreted as an artefact of dragging upon felling. However, the presence of clear (if weakly) demarked first and second order branches that are both aligned with and fit the size profile of other branches in the frond, renders this interpretation unlikely. Laflamme *et al.* (2007) documented the parallel-sided morph from Lower Mistaken Point on the Avalon Peninsula, but do not describe any form of connecting region, with branches connecting directly to the holdfast (their fig. 6I–J), providing further support that this area may not be a 'stem'. Taken together with the variability in presence and appearance of branches in the connecting region in bedding plane populations of specimens in Newfoundland, the connecting region is likely to represent an artefact of specimen twisting upon felling and burial. Twisting would not necessarily affect branch preservation in more apical regions but could result in the apparent absence or poor preservational fidelity of branches closer to the base of the frond.

The base of the *Charnia masoni* frond thus appears to reflect an area with considerable morphological variation, perhaps resulting from taphonomic, environmental, and/or biological factors. The proportional length of this region is variable even across specimens of a similar size from the same bedding plane (Fig. 4; Table 1). Some of this intraspecific variation may suggest a hitherto unrecognized plastic element to *C. masoni* growth and morphology, and a potential capacity to respond to local environmental factors (e.g. neighbour competition or nutrient availability) by differential growth (cf. Hoyal Cuthill & Conway Morris 2017; Kenchington & Wilby 2017).

None of the specimens examined show evidence for an internal stalk running along the length of the organism, such as that seen in other rangeomorphs (e.g. *Avalofractus abaculus*, Narbonne *et al.* 2009, or *Rangea schneiderhoehni*, Vickers-Rich *et al.* 2013; Sharp *et al.* 2017). Stalk-like structures observed in our investigations are interpreted as the effaced remains of first order branch margins (Fig. 2C, D). Indeed, space constraints (highlighted by Grazhdankin 2004*a*) mean that the presence of such a stalk in *Charnia masoni* is unlikely. An alternative scenario involves the central axis in *C. masoni* being constructed by successively stacked lateral branches (schematically represented in Fig. 11D), conferring a sympodially organized central axis (as opposed to the monopodial arrangement present in *Avalofractus* or *Rangea*). We note here the distinctive nature of the basal-most branches in *C. masoni*, which differentiate directly from the holdfast (Dunn *et al.* 2018*a*). However, we acknowledge that it currently remains difficult to differentiate between these two possible axial arrangements based on the available evidence.

Previous taxonomic schemes for rangeomorphs have placed emphasis on an internal stalk (Laflamme &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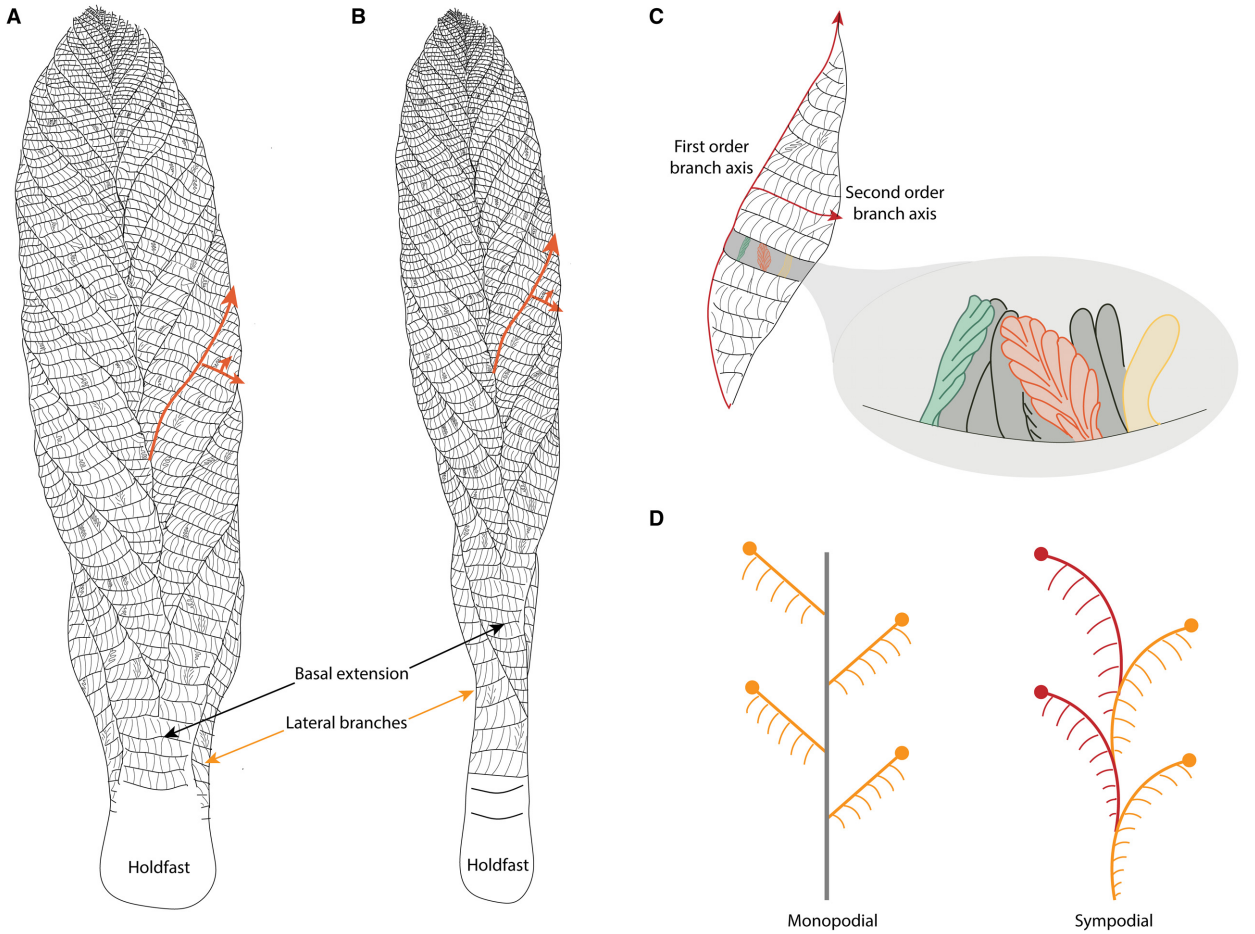


**FIG. 11.** Morphological model of *Charnia masoni*. A–B, Charnwood-like and parallel-sided morphotypes of *Charnia masoni*, respectively; orange arrows indicate the orientation of the branch axes up to third order; twisting of central axis is illustrated in B. C, observed variation in third and fourth order branch organization; the orange branch is displayed and unfurled (see Fig. 1C), the green branch is rotated and unfurled (see Fig. 3G) and the yellow branch is undivided and furled (see Fig. 6E); terminology after Brasier *et al.* (2012); red arrows indicate the first order branch axis (oriented apically) and the second order branch axis (oriented laterally). D, monopodial and sympodial central axial arrangements; monopodial growth is characterized by lateral branches emerging from a single central axis, while sympodial growth is characterized by successively stacked lateral branches, without a separate central axial structure (e.g. Berking 2006).

Narbonne 2008; Brasier *et al.* 2012) and whether it is exposed or concealed. Narbonne *et al.* (2009) illustrated a structure they interpreted as an internal stalk in a *Charnia*-like frond. However, this structure could alternatively be explained by sedimentary or taphonomic processes (Grazhdankin & Seilacher 2005; Brasier *et al.* 2013) and, given the very small number of such known examples, we do not consider it a compelling morphological feature. Stalks (as opposed to stems) are assumed but not demonstrated to be present in several other rangeomorphs including the uniterminal *Beothukis mistakensis* and *Beothukis plumosa*, or the biterminal rangeomorph genus *Fractofusus.* Some extant frondose organisms (e.g. hydrozoan cnidarians) are known to display variation in axial arrangement within a clade (e.g. Berking 2006) and so the idea that, even if they are a monophyletic group, all rangeomorphs must share similar axial arrangements may be erroneous.

**FIG. 10.** *Charnia masoni* from the Winter Coast of the White Sea, Russia. A–B, PIN 3993-7023; rotated and furled third order branches evenly spaced at the base of a second order branch but oriented medially at the apex. C, PIN 3993-7023; clean separations between second order branches, and variation in their width of separation, indicate that the second order branches were probably discrete units each with its own boundary wall (rather than a shared wall with adjacent second order branches). D–E, PIN 3993-7025; furled, distally inflated fourth order branches (expanded in E) with no further subdivisions visible. Scale bars represent 10 mm.

The morphology of the holdfasts in *Charnia masoni* can vary markedly between different specimens (Grazhdankin *et al.* 2008, fig. 2A; Wilby *et al.* 2015, fig. 4), ranging from circular to diamond in shape. This variation could represent either true biological or taphonomic variation (Burzynski *et al.* 2017), or a combination of the two. The most parsimonious scenario is that differing depths of holdfast burial account for the majority of observed variation in our studied populations.

The redescription of *Charnia masoni* allows construction of a new model for its *in vivo* anatomy (Fig. 11). The organism was attached to the sediment by a bulbous holdfast and was constructed of a series of stacked first order branches arranged in two rows, which may have been derived successively from a sympodial central axis, or from a cryptic monopodial axis. Each first order branch had an apical axis from which a series of second order branches emerged laterally. Third order branches were attached to the second order branch axes and were oriented apically. Variation in both original anatomy and preservation near the base of the organism results in the variable presence or absence of both a basal extension, and the lateral branches.

## CONCLUSIONS

Evaluation of the morphology of *Charnia masoni* from three late Ediacaran assemblages (Charnwood Forest, Newfoundland, and the White Sea) enables assembly of an emended model of morphology for this organism, demonstrating greater levels of intraspecific variation than have previously been documented. *C. masoni* specimens from the different localities are comparable in morphology but show features that cannot easily be reconciled with previous rangeomorph taxonomic regimes, and potentially fall outside the current definition of Rangeomorpha. Our study reveals that certain characters previously proposed as taxonomically informative, such as the displayed/undisplayed, furled/unfurled nature of branches, are fallible at higher branch orders. We provide an emended diagnosis of *Charnia masoni* to take account of the novel features and variation described herein (see below).

A more detailed understanding of anatomy must necessarily precede phylogenetic interpretation, since organisms must be interpreted as the sum of all their parts. Our novel interpretation of anatomy in *Charnia masoni*, an organism that is among the most widely studied of the Ediacaran macrobiota, illustrates the potential for obtaining significant amounts of new information from global-scale, population-wide studies of well preserved Ediacaran specimens.

## SYSTEMATIC PALAEONTOLOGY

### Genus CHARNIA Ford 1958

*Emended diagnosis.* Frond uniterminal, comprising two rows of non-conjoined first order branches arranged alternately along a central axis, presenting as a zig-zag medial suture. First order branches typically show proximal inflation, whereas (non-conjoined) second-order units show moderate-to-medial inflation. All first to fourth order branches are aligned in subparallel series. Second order branches are oriented basally, whereas first and third order branches are oriented apically. First order branches comprise rangeomorph elements that are rotated and undisplayed, while second order branches are comprised of rangeomorph elements that may be rotated and either furled or unfurled. There is variation in the presentation of third and fourth order rangeomorph branch elements, which can be displayed and unfurled, displayed and furled, undisplayed and furled, or undivided. A basal disc is present in some specimens.

*Type species.* *Charnia masoni* Ford, 1958.

### *Charnia masoni* Ford, 1958

| | |
|---|---|
| v* 1958 | *Charnia masoni* Ford, p. 212, pl. 13, fig. 1. |
| ? 1959 | *Charnia* sp.; Glaessner, p. 1472, text-fig. 1b. |
| ? 1959 | *Rangea?*; Glaessner, *in* Glaessner & Daily, p. 387, pl 46, fig. 2. |
| 1961 | *Charnia* sp.; Glaessner, p. 75, text-fig. |
| 1962 | *Charnia* sp.; Glaessner, pp 484–485, pl. 1, fig. 4 (*non* fig. 5). |
| 1962 | *Charnia masoni*; Ford, fig. 4 (*non* fig. 5). |
| 1966 | *Rangea grandis*; Glaessner & Wade, p. 616, pl. 100, fig. 5. |
| 1972*a* | *Rangea sibirica*; Sokolov, pl. I, fig. 3. |
| 1972*b* | *Rangea sibirica*; Sokolov, p. 50. |
| 1973 | *Glaessnerina grandis*; Germs, p. 5, fig. 1D. |
| 1976 | *Charnia* ex gr. *masoni*; Sokolov, p. 141. |
| 1977 | *Charnia* ex gr. *masoni*; Sokolov, p. 441. |
| 1978 | *Charnia masoni*; Fedonkin, fig. 3 (9). |
| 1979 | *Charnia masoni*; Glaessner, fig. 12 (3). |
| 1979 | *Glaessnerina sibirica*; Glaessner, fig. 12 (1). |
| 1981*a* | *Charnia masoni*; Fedonkin, p. 66, pl. 3, figs 5, 6; pl. 29, fig. 1. |
| 1981*a* | *Zolotytsia biserialis*; Fedonkin, p. 67–68, pl. 3, fig. 7. |
| 1981*b* | *Charnia masoni*; Fedonkin, p. 100. |
| 1981 | *Charnia masoni*; Sokolov & Brekhovskikh, p. 3. |
| 1981 | *Glaessnerina grandis*; Glaessner & Walter, fig. 6.11 (C). |
| 1983*a* | *Charnia masoni*; Fedonkin, fig. 37. |
| 1983*b* | *Charnia masoni*; Fedonkin, pl. 1, fig. 1. |

1983 *Charnia masoni*; Sokolov & Fedonkin, p. 13, fig. 9.
1984 *Charnia masoni*; Sokolov, p. 6, fig. 1.
1984 *Charnia masoni*; Glaessner, fig. 2.21 (A).
1984 *Glaessnerina sibirica*; Glaessner, fig. 2.21 (D).
1984 *Glaessnerina grandis*; Glaessner, fig. 2.21 (C).
1984 *Charnia masoni*; Sokolov & Fedonkin, fig. 3 (f).
1984 *Charnia* cf. *C. masoni*; Glaessner, fig. 2.21 (B).
1985 *Charnia masoni*; Fedonkin, p. 99, pl. 12, fig. 4; pl. 13, figs 2–4.
1985 *Charnia* cf. *C. masoni*; Jenkins, fig. 7 (C).
1985 *Charnia masoni*; Jenkins, fig. 7 (B).
1987 *Charnia masoni*; Fedonkin, pl. 15.
1987 *Glaessnerina grandis*; Preiss, p. 310, fig. E.
1990 *Charnia masoni*; Fedonkin, fig. 1 (D).
1992 *Charnia masoni*; Fedonkin, fig. 28–30.
1992 *Charnia masoni*; Runnegar & Fedonkin, fig. 7.5.5 (A), fig. 7.5.10 (A).
1994 *Charnia masoni*; Fedonkin, fig. 2 (A, B).
v* 1995 *Charnia grandis*; Boynton & Ford, p. 168, fig. 1.
1996 *Glaessnerina grandis*; Jenkins, p. 35, fig. 4.1.
v 1997 *Charnia masoni*; Grazhdankin & Bronnikov, p. 794, fig. 2 (a, d).
? 1998 *Charnia masoni*; Nedin & Jenkins, p. 315, fig. 1.
1999 *Charnia grandis*; Ford, p. 231, fig. 3.
v 2000 *Charnia*; Martin *et al.*, fig. 4 (A).
v 2004*a* *Charnia*; Grazhdankin, p. 207, fig. 2.
2005 *Charnia masoni*; Narbonne *et al.*, p. 28, pl. 1L.
v 2005 *Charnia*; Grazhdankin *et al.*, fig. 3 (d).
v 2007 *Charnia masoni*; Laflamme *et al.*, p. 243, fig. 4A–J.
v 2007 *Charnia* sp.; Fedonkin *et al.*, p. 128, fig. 232 (*partim*).
v 2007 *Charnia* cf. *masoni*; Fedonkin *et al.*, p. 145, fig. 276 (*partim*).
v 2007 *Charnia* cf. *masoni*; Fedonkin *et al.*, p. 160, 165, figs 304, 314 (*partim*).
v 2007 *Charnia masoni*; Fedonkin *et al.*, p. 186, fig. 354.
2008 *Charnia masoni*; Hofmann *et al.*, p. 17 (*partim*), fig. 13.1.
v 2008 *Charnia grandis*; Hofmann *et al.*, p. 18, fig. 14.
v 2008 *Charnia masoni*; Grazhdankin *et al.*, p. 804, fig. 2A.
v 2009 *Charnia masoni*; Bamforth & Narbonne, p. 907, fig. 7.5.
v 2011 *Charnia masoni*; Wilby *et al.*, pp 656–657 (*partim*), figs 2A, 3A.
v 2011 *Charnia masoni*; Grazhdankin, fig. 3 (a–d).
v 2012 *Charnia masoni*; Liu *et al.*, p. 397, figs 4B, 5A.
v. 2013 *Charnia* aff. *masoni*; Liu *et al.*, p. 24, fig. 1D.
v 2013 *Charnia masoni*; Liu *et al.*, p. 24, fig. 2A–D.
2013 *Charnia* sp.; Gehling & Droser, p. 449, fig. 2Q.
v 2014 *Charnia masoni*; Grazhdankin, p. 271 fig. 2.3.
v 2015 *Charnia masoni*; Wilby *et al.*, p. 20, fig. 2.1,3,6, fig. 2.2,4, fig. 2,5.
v 2015 Incomplete frond; Wilby *et al.*, p. 20, fig. 2.8.
v 2015 *Charnia masoni*; Liu *et al.*, p. 1361, fig. 2D.
v 2016 *Charnia masoni*; Liu *et al.*, p. 5 *(partim)*, fig. 3D.
v 2017 *Charnia masoni*; Antcliffe *et al.*, p. 27, fig. 4E.
v 2018*a* *Charnia masoni*; Dunn *et al.*, p. 5, fig. 1E, p. 7, fig. 3.

*Diagnosis.* As for the genus.

*Remarks.* We do not consider the described variation between specimens of *Charnia masoni* from Charnwood, Russia and Newfoundland to be taxonomically significant. Following recent taxonomic discussions on rangeomorphs, we consider all studied specimens to at least belong within the same genus (cf. Liu *et al.* 2016; Kenchington & Wilby 2017). Determination of whether the specimens represent morphs of the same species, or separate species, is more challenging. Where there is variation in multiple continuous characters within Ediacaran taxa, it has been proposed that this would be sufficient to indicate species level differences (Liu *et al.* 2016), depending on the nature and extent of this variation (Kenchington & Wilby 2017). However, when considering morphs from different localities, it can be extremely difficult to distinguish between taxonomic and intraspecific variation (Kenchington & Wilby 2017). Although both parallel-sided (Newfoundland) and ovate (Charnwood, White Sea) morphs of *C. masoni* may be present on individual surfaces (e.g. Fig. 7, from Bed LC6), such occurrences are rare and there is typically one numerically dominant morph.

If further variation (categorical or continuous *sensu* Kenchington & Wilby 2017) is described in these morphs, we would consider it appropriate to reassess these conclusions. Indeed, if variation in discrete characters is identified, then it may be appropriate to erect a new genus.

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## DATA ARCHIVING STATEMENT

A reflectance transformation image of the *Charnia masoni* holotype (LEIUG 2328) is available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.fg14s2r

*Editor*. George Sevastopulo

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Report

Current Biology

# Modularity and Overcompensatory Growth in Ediacaran Rangeomorphs Demonstrate Early Adaptations for Coping with Environmental Pressures

## Highlights

- A new multifoliate rangeomorph from Charnwood Forest, *Hylaecullulus fordi*, is described
- *Hylaecullulus* shows eccentric branches, interpreted as overcompensatory growth
- This growth shows that *Hylaecullulus* was modular and able to recover from damage
- Modularity in rangeomorphs enabled them to achieve large body sizes

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## In Brief

Charlotte et al. describe a new rangeomorph (*Hylaecullulus fordi*) from the Ediacaran of Charnwood Forest, UK. These fossils show evidence of overcompensatory growth, demonstrating that *H. fordi* was able to recover from damage. It is evidence of biological modularity in rangeomorphs, a construction shared by the first clades to achieve large size.

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Report

# Modularity and Overcompensatory Growth in Ediacaran Rangeomorphs Demonstrate Early Adaptations for Coping with Environmental Pressures

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## SUMMARY

**The first known diverse, complex, macroscopic benthic marine ecosystems (late Ediacaran, ca. 571–541 Ma) were dominated by the Rangeomorpha, an enigmatic group of extinct frondose eukaryotes that are candidate early metazoans [1, 2]. The group is characterized by a self-similar branching architecture that was most likely optimized for exchange, but nearly every other aspect of their biology is contentious [2–4]. We report locally enhanced, aberrant growth (''eccentric branching'') in a stalked, multifoliate rangeomorph—*Hylaecullulus fordi* n. gen., n. sp.—from Charnwood Forest (UK), confirming the presence of true biological modularity within the group. Random branches achieve unusually large proportions and mimic the architecture of their parent branch, rather than that of their neighbors (the norm). Their locations indicate exceptional growth at existing loci, rather than insertion at new sites. Analogous overcompensatory branching in extant modular organisms requires the capacity to orchestrate growth at specific sites and occurs most frequently in response to damage or environmental stress, allowing regeneration toward optimum morphology (e.g., [5–7]). Its presence in rangeomorphs indicates a hitherto unappreciated level of control to their growth plan, a previously unrecognized form of morphological plasticity within the group, and an ability to actively respond to external physical stimuli. The trait would have afforded rangeomorphs resilience to fouling and abrasion, partially accounting for their wide environmental tolerance, and may have pre-adapted them to withstand predation, weakening this argument for their extinction. Our findings highlight that multiple, phylogenetically disparate clades first achieved large size through modularity.**

## RESULTS

### Systematic Paleontology

#### *Material*

Six well-preserved specimens, all preserved in lateral aspect (Figure 1), from the top surface of a single bedding plane (Bed B of [8]) in the Bradgate Formation, Maplewell Group, Charnwood Forest, UK (Figure S1). Two co-occurring, poorly preserved specimens (GSM106012 and GSM106034; Figure S2) are also assigned to the genus. All specimens are current aligned with the other fossils on the surface and are preserved as low epirelief impressions. Master molds and casts are housed at the British Geological Survey, Keyworth, UK (nos. GSM105875, GSM105957, GSM105958, GSM105959, GSM106040, and GSM106112); original specimens remain *in situ*. Reflectance transformation imaging (RTI) [9, 10] files of specimens GSM105875, GSM106040, and GSM106112 are available (resource listed in the STAR Methods). For a description of rangeomorph terminology, see [4].

#### *Genus* Hylaecullulus *gen. nov.*

Genus *Hylaecullulus* gen. nov.

Type species *Hylaecullulus fordi* sp. nov. by monotypy.

The plastotype is designated as GSM105875 (Figure 1A); GSM106040 (Figure 1C) and GSM106112 (Figure 1E) are designated as plastoparatypes.

*Etymology*. Named for the goblet-like shape of the organism (Greek *Cullulus*, a goblet) and its occurrence in Charnwood Forest (Greek *Hylaeos*, meaning from the woods)

*Diagnosis*. Rangeomorph comprising a disc and similarly sized crown, connected by a straight and proportionally long and narrow stem. The disc typically has several concentric rings and frequently includes a triangular feature at its junction with the stem. The stem is of uniform width along its length and is longer than the crown. The crown has a sub-circular outline and is multifoliate, comprising numerous folia emanating from a single location at the distal end of the stem. The folia are displayed, unfurled or furled, and unconstrained and show distal inflation. Primary branches are typically displayed, furled, radiating, and unconstrained and show proximal inflation; unfurled branches may be locally present. Secondary branches are displayed, furled, radiating, and unconstrained and show distal inflation. Tertiary branches are displayed, furled, and constrained and show slight

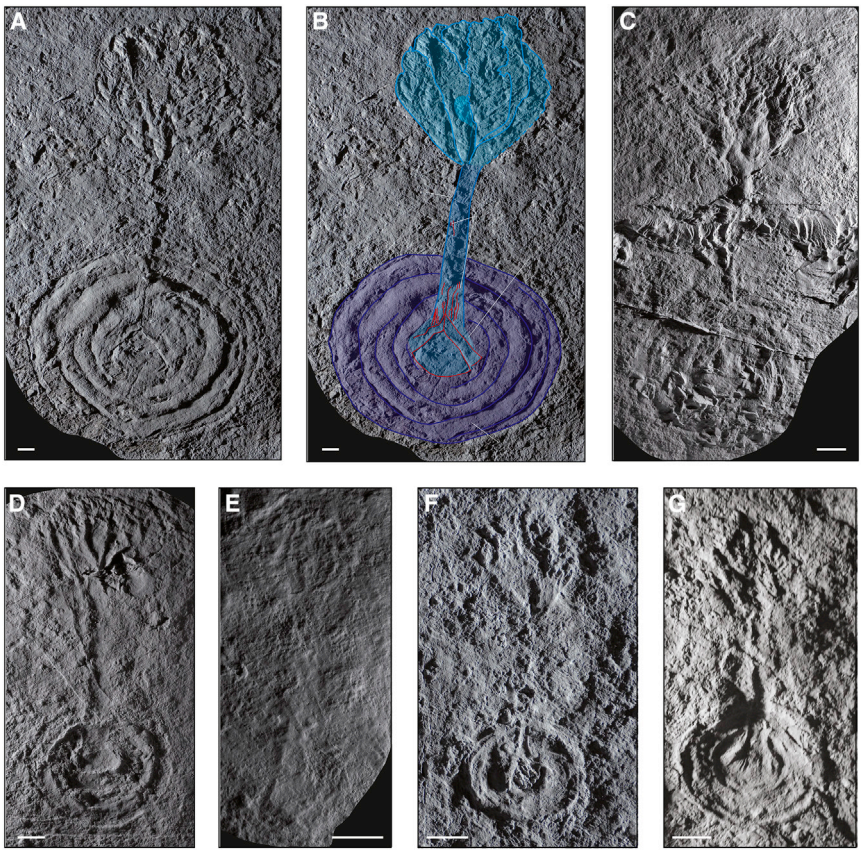


**Figure 1. Specimens of *Hylaecullulus fordi* from Charnwood Forest**

(A) GSM105875 (mold), the plastotype and largest known example.

(B) Interpretive overlay (up to folium level detail) of GSM105875. The dark blue area is the holdfast disc, with dark blue lines outlining its internal rings. Medium blue is its stem, with red lines defining the "lineations" and "triangle." Bright blue outlines the folia.

(C) Plastoparatype GSM106040 (mold).

(D) GSM105959 (cast).

(E) Plastoparatype GSM106112 (cast).

(F) GSM105957 (cast), the smallest well-preserved example.

(G) GSM 105958 (cast).

Scale bars, 2 cm. All molds and casts are held at the British Geological Survey, Keyworth. The interpretative overlay was digitized from a camera-lucida interpretation. Stratigraphic setting is shown in Figure S1, and additional specimens are shown in Figure S2.

radiation and slight distal inflation. Branch axes of all orders are concealed, and opposing ones are offset along the length of their host branch. The folia and first- and second-order branches, at least, may bear "eccentric branches" at any point along their length; these conform to the branching pattern of the host branch, rather than their neighboring branches of the same order.

## Hylaecullulus fordi *sp. nov.*

*Hylaecullulus fordi* sp. nov.

2011 "dumbbell-like taxon," "dumbbell-like frond" [8] p. 656, Figures 2D and 4.

2012 "multi-ringed impression," "unnamed species" [11], Figure S3.

2017 "dumbbells" [4], Figure S1A.

*Diagnosis*. As per genus.

*Etymology*. Named for Trevor Ford, in recognition of his contribution to Ediacaran paleontology.

*Description*. The heights of known specimens, from the base of the stem (i.e., center of the disc) to the distal margin of the crown, range from 7.6 cm to 37.6 cm (Table S1). Disc diameter ranges from 2.7 cm to 27 cm and increases proportionally with total height. The disc has a well-defined outer margin and a variable number (two to five) of prominent concentric rings. The stem is straight and of uniform width, except at its base, where it expands abruptly into a triangular structure to meet the disc, and it comprises between 58% and 69% of the total height of the organism. The triangular structure is approximately one-third of the width of the disc and overlays the disc. The stem of the largest specimen (GSM105875) displays fine, closely spaced, parallel lineations along much of its length, interpreted as biostratinomic artifacts (Figures 1A and 1B; *cf*. [12]).

The crown is broadly circular in outline, with a well-defined, scalloped distal margin (Figure 1B). It is slightly wider than it is high, and its width has an almost 1:1 correlation ($R^2 = 0.9737$) with that of the disc. Its shape is maintained throughout known ontogeny. The crown consists of numerous partially overlapping folia [4], all emanating from the terminus of the stem. Five folia are visible in the majority of specimens (Figure 1), but only four are clearly preserved in the smallest (GSM105957). Additional (taphonomically overlying) folia are suggested by the frond's scalloped distal margin. The organism is interpreted to have had a goblet-shaped morphology (Figure S3)—the functional significance of its morphology is discussed in the STAR Methods (under Method Details).

At least three orders of branching can be resolved within the folia of the best-preserved specimens (Figures 2 and 3; Table S2), with a fourth suggested in the holotype (GSM105875; Figure 3A). Folia are displayed and unconstrained, show median-distal inflation, and are unfurled; in three specimens (GSM105959, GSM105957, and GSM105957; Figures 1D, 1F, and 1G), folia are locally furled at their bases. Primary branches are displayed, furled, radiating, and unconstrained and show moderate proximal-median inflation. In two specimens (GSM105875 and GSM106040), some primary branches are unfurled. Secondary branches are displayed, furled, radiating, and unconstrained and inflate moderately distally. Tertiary branches are displayed, furled, and constrained and show moderate radiation and slight distal inflation.

Eccentric branches occur on folia, primary branches, and (rarely) secondary branches of the three best-preserved specimens (Figure 3); these include the two largest individuals (GSM105875 and GSM106040) and a comparatively small one (GSM106112). Eccentric branches are oversized relative to their

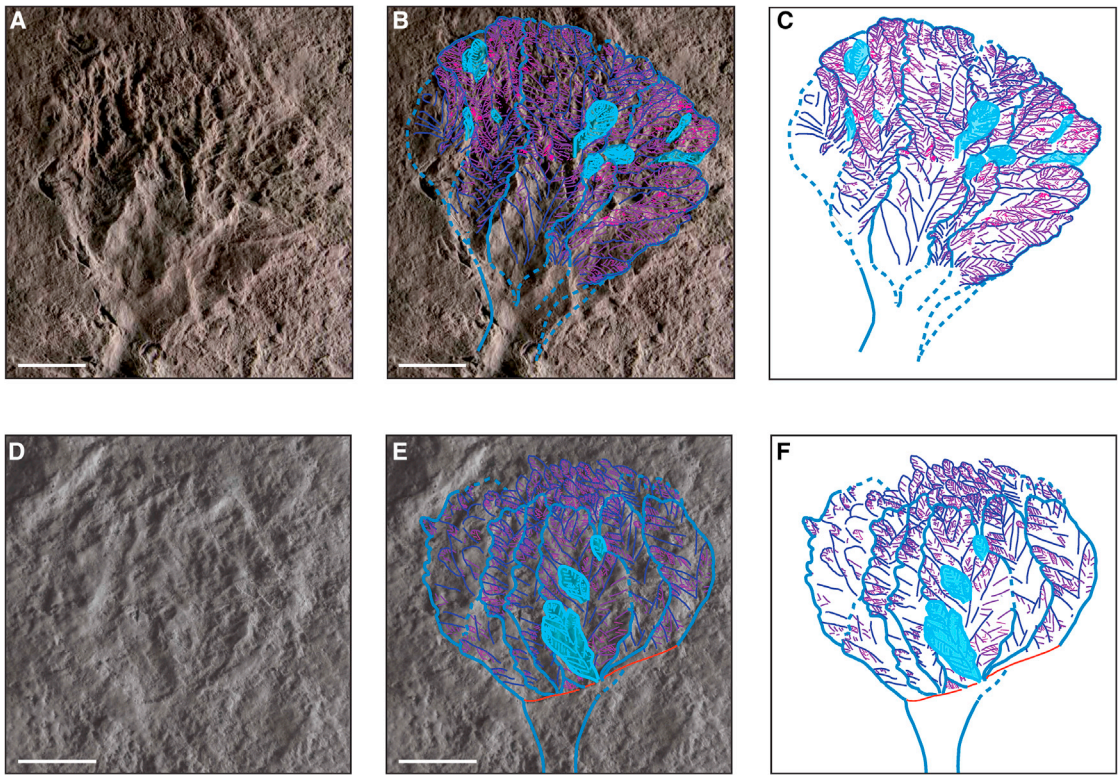


**Figure 2. Detailed Branching Architecture of *Hylaecullulus fordi***

(A–C) GSM106040 (cast). A close-up of (A) is shown in (B), and an interpretative overlay of (B) is shown in (C).

(D–F) GSM106112 (cast). A close-up of (D) is shown in (E), and an interpretative overlay of (E) is shown in (F).

Scale bars, 2 cm. All casts are housed at the British Geological Survey. Interpretative overlays were digitized from camera-lucida interpretations; see STAR Methods.

neighbors on the same host branch but occupy a normal branch position (rather than, for example, representing branches of a lower-order poking through; shown schematically in Figure S3). In all cases, their branching pattern mimics that of the host branch, rather than that of their neighbors (Figure 2). Multiple examples are present in all three specimens (Figures 2 and 3). Eccentric branches may occupy any position along the host branch and within the crown, with no clear bias for either distal or proximal end (Figures 2 and 3). Clustering of eccentric branches is apparent on secondary branches, is less common on primary branches, and has not been observed on folia (Figures 2 and 3).

## DISCUSSION

The late Ediacaran (ca. 571–541 Ma) was an interval of pronounced anatomical and ecological innovation, exemplified by the appearance of diverse assemblages of macroscopic, soft-bodied organisms (e.g., [1, 3]). Collectively referred to as the Ediacaran biota, these organisms are distinct from earlier macroscopic algae (see [2]) and may offer insights into the origination and early evolution of major clades [1], the assembly of benthic marine ecosystem (see [3]), and the nature of the Ediacaran-Cambrian biotic transition [13]. The Rangeomorpha [14] are an important component of the Ediacaran biota, dominating early, deep-marine settings [3]. Their phylogenetic placement is contentious, but they have recently been placed within the Metazoa on the basis of their developmental biology [2]. They are characterized by fronds with a self-similar pattern of alternate branching, resolvable over up to four orders of subdivision; details of their branching architecture underpin their taxonomy and phylogeny [3, 4, 15–17]. Many taxa also possess a holdfast and a stem that acted to lift the frond clear of the substrate [18, 19]. Their precise mode of feeding has generated particular interest because of its potential phylogenetic and ecological implications (e.g., [3]), but there is general agreement that their fronds functioned as exchange surfaces [3, 4, 20, 21].

The preservation of rangeomorphs as external molds [22] has necessarily meant that many aspects of their biology and ecology are inferred from indirect evidence, particularly from their growth and developmental characteristics [2]. A modular organization has been assumed based on their self-similar branching architecture [17, 20, 23], but supporting evidence for their branches (modules) having had developmental or physiological independence from one another [24, 25] has been lacking.

### Rangeomorph Construction

Rangeomorphs are considered to be fundamentally similar to each other, with relatively minor deviances from a common growth strategy accounting for anatomical differences (e.g., [17]). The

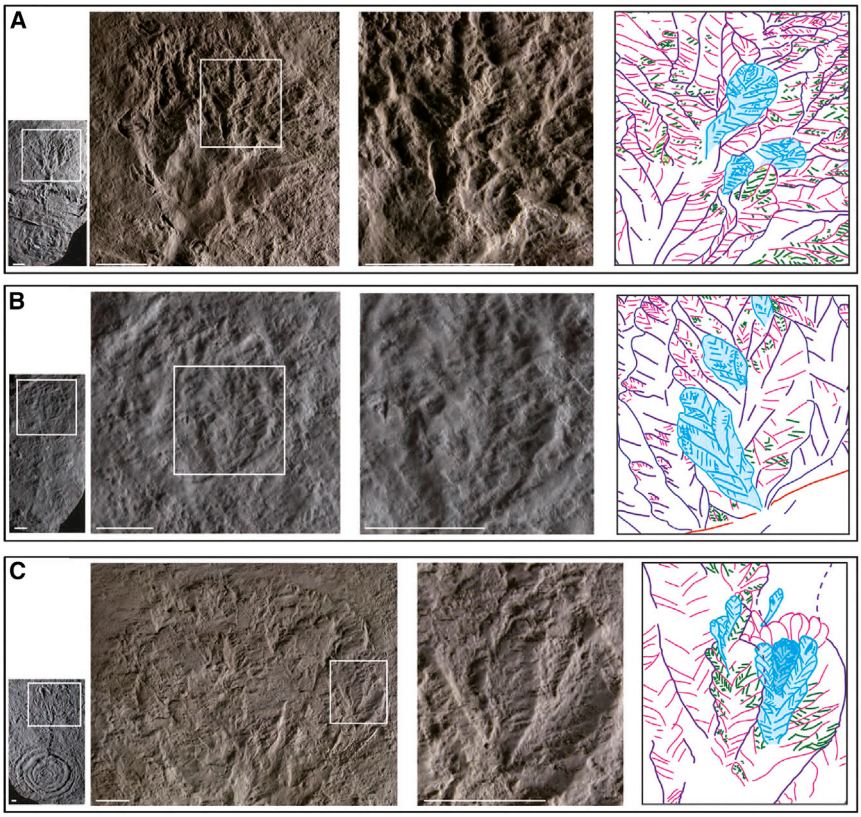


**Figure 3. Eccentric branching in *Hylaecullulus fordi***

Increasingly higher-magnification views of the outlined boxed areas; the final image is an interpretative overlay (digitized from camera-lucida drawings) of the penultimate image. GSM106040 (cast) (A), GSM106112 (cast) (B), and GSM105875 (cast) (C) are shown. Scale bars, 2 cm. All casts are housed at the British Geological Survey, Keyworth. An artist's reconstruction is shown in Figure S3, and a comparison to *Bradgatia* is shown in Figure S4.

morphology of *Charnia masoni* has been used as a model for rangeomorph growth. New branches differentiated from a generative zone at or near the distal tip on alternate sides of a central axis and subsequently inflated [26]. The relative dominance of differentiation versus inflation varies between taxa (e.g., [2, 15, 27]) and, in certain species at least, varied during ontogeny and/or in response to environmental pressures (see [28]). Minor deviations from this model are poorly recorded but, where identified, are typically attributed to taphonomic effects and intra-specific variation (see [2, 4]). However, there is suggestion that the growth strategy of *Charnia* (and so perhaps other rangeomorphs) was more complex than previously envisaged [2].

Eccentric branching subverts known rangeomorph growth programs and indicates a hitherto unrecognized level of morphological plasticity (see [28]). It is distinct from the subsidiary branching recognized in *Bradgatia lindfordensis* [15] and the subsidiary frondlets in *Fractofusus misrai* [27], both of which record insertion at additional growth loci between normal branches, rather than aberrant, enhanced growth at existing sites (as in eccentric branches). Consequently, we do not consider eccentric branching to be part of pre-determined growth architecture, but rather deviant growth. We find no instance of eccentric branching in known unifoliate fronds: none was found in well-preserved specimens of *Charnia masoni* from Charnwood Forest [28] or in *Beothukis*, *Vinlandia antecedens*, and *Trepassia wardae* from Newfoundland [15, 23]. However, we recognize eccentric branching in other multifoliate fronds—*Bradgatia* and *Primocandelabrum* [4]—from the same bedding-plane surface as *H. fordi*. Given the apparently random distribution of eccentric branches within the crown (Figure 2), we consider them to most likely be a response to damage or abrasion, rather than growth in response to, for example, changing nutrient concentrations (*cf*. [21])

## Implications for Rangeomorph Biology

New growth in response to damage which outpaces normal growth—termed "overcompensatory" growth—is a phenomenon peculiar to truly modular organisms. A module is a group of elements whose interactions occur preferentially within the group, such that the activity of elements within a module may depend little on elements outside of it [24, 25]. The expression of overcompensatory growth varies between groups. Some gorgonian octocorals exhibit a remarkably similar morphological response to *Hylaecullulus fordi*, with branches reverting to higher order states and growing faster than normal [29]. Similar peripheral damage in plant leaves does not elicit similar results, and damage to the central stem does not result in overgrowth or repair, but rather the specification of new apical or sub-apical generative zones, with multiple new shoots borne from the vascular cambium (e.g., [30]). Bryozoans, which are the only extant colonial bilaterians that commonly produce an arborescent form, may repair the original structure or show little growth response (e.g., [31]) but show no overcompensatory response [5]. Regeneration in fragmented graptoloid colonies (monograptids) is generally marked by an abrupt change in thecae size and shape and by the subsequent iteration of uniform thecae resembling typical distal thecae, rather than the normal astogenetic gradient of morphologies; where regeneration has taken place without a sicula (i.e., from a distal fragment), it additionally leads to development of a new branch (growth pole) in the opposing direction [32]. Rarely, the regenerated portion may show an abbreviated astogenetic succession [33]. Algae are less predictable, although broadly similar outcomes to eccentric branches may be generated. In the coenocytic chlorphyte *Caulerpa*, for example, rather than only branches appearing eccentric, complete fronds (including stem) can emerge from the middle of another frond (Figure 4).

The clustering of eccentric branches in *Hylaecullulus fordi*, as well as their restriction to specific orders of host branch, strongly

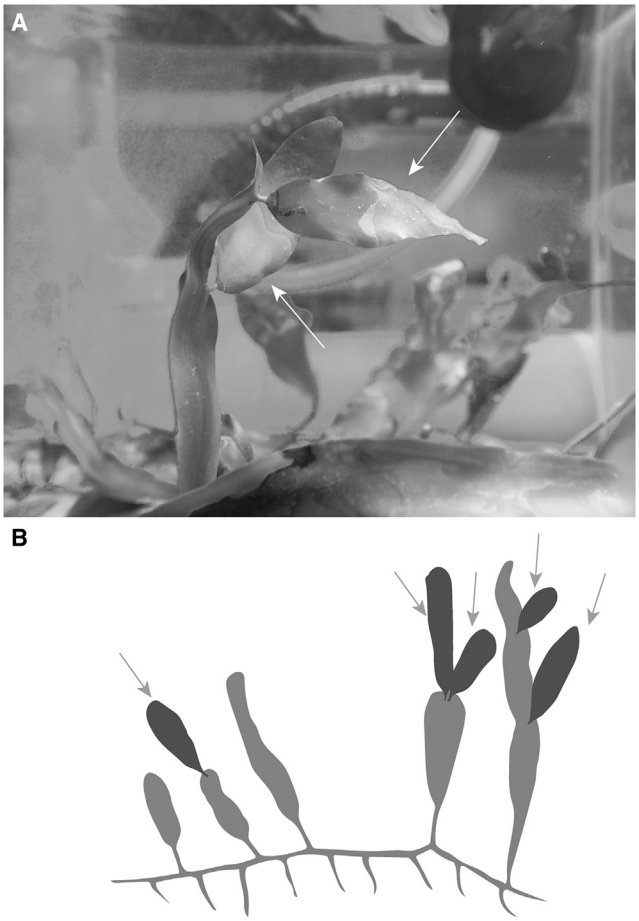


**Figure 4. Aberrant Growth in the Chlorophyte *Caulerpa***

(A) *Caulerpa prolifera* with aberrant fronds (frond emerging directly from another frond, as opposed to from the basal stolon) arrowed.

(B) A schematic of *Caulerpa prolifera* illustrating the variability of the aberrant fronds (arrowed).

suggests an ability to target growth, and also perhaps that the pattern of higher order branches was fixed at inception—they did not have the capacity for eccentricity. These differences between branch orders contradict previous interpretations of simple and iterative growth in rangeomorphs (see [2, 17, 23]). Regardless of the trigger stimulus, the capacity to orchestrate enhanced growth at specific sites indicates the ability either to turn on local production of growth factor or to target its delivery from a remote point. Both mechanisms indicate a greater level of control and complexity to the rangeomorph growth program than previously assumed: while locally controlled production of growth factor would suggest greater module autonomy, targeted delivery would suggest a high level of physical interconnectedness between modules. Based on the available specimens of *H. fordi*, there is currently no way to distinguish between these two alternatives, and previous reports of an unspecified internal, semi-rigid, organic skeleton within rangeomorphs [23] have subsequently been dismissed as taphonomic artifacts (see [22]). Consequently, the degree to which resources may have been shared between modules within a frond remains unknown. That individual branches within multifoliate fronds display overcompensatory growth, reverting to a lower-order branch architecture, and that they were able to respond and adapt independently to their environment indicates, for the first time, that they constituted true biological modules.

The apparent restriction of eccentric branching to multifoliate forms suggests that phenotypic plasticity, and potentially the presence of true modularity, varied within rangeomorphs, as it does in many extant groups (e.g., [34]). The absence of eccentric branching in *Charnia* would seem to suggest tighter controls on the autonomy of individual branches, consistent with its constrained architecture [2, 28]. Eccentric branching may even have been selected against in unifoliate rangeomorphs because such branches would distort the outline of the frond and impact its efficiency (*cf*. [19]). In a similar vein, branching style and overall morphology of octocorals varies according to their degree of module integration (coloniality [34]). The oldest known rangeomorphs are unifoliate, appearing several million years before multifoliate forms [3, 35]. Hence, we speculate that the modularity in multifoliate forms may be derived. Any such move to true (or at least overt) modularity could be considered conceptually comparable to the independent shifts to coloniality (and thus modularity) seen in extant invertebrate groups. For example, the plesiomorphic condition for crown-group cnidarians was most likely unitary, but successive transitions to colonialism are known in both the Octocorallia and the Hexacorallia [36]. Colonial bilaterian groups (e.g., bryozoans, entoprocts, or rotifers) developed from unitary bilaterian ancestors [37, 38]. Colonies are considered to develop by the weakening of zooid individuality in order to strengthen colony identity, conferring advantages to the colony as a whole [39]. Rangeomorphs could plausibly have developed modularity by greater integration (as with metazoans) or by the relaxation of integration and appearance of semi-autonomy (as with plants and algae); it is not yet possible to discriminate which.

Modularity may bestow a number of ecological advantages, including increased overall size and complexity with limited changes in surface area to volume ratios; enhanced feeding efficiency, given the greater potential for at least one module being in an optimum position; greater plasticity and, consequently, adaptability; and increased resilience to damage, with the loss of one module not necessarily compromising the entire organism [40]. It is also a means of achieving large body size. Indeed, the three earliest groups to have achieved macroscopic size—algae, fungi, and now rangeomorphs—did so through modularity. That rangeomorphs were able to respond to environmental stressors has significant ramifications for understanding of their ecology. Targeted growth in response to damage is a highly beneficial trait in extant sessile organisms, enabling them to maintain their optimum form and to better cope with environmental constraints [6, 7, 29]. By extension, this trait would most likely have proved particularly advantageous for multifoliate rangeomorphs, whose unconstrained, overlapping branches would have been prone to abrasion by neighboring ones and susceptible to fouling by suspended sediment. It potentially helps explain their successful invasion of both deep-water environments and shallower, more energetic, settings [3, 28]. Such regenerative capabilities may have potentially acted as a pre-adaptation to withstanding predation, one of several proposed drivers of the extinction of Ediacaran organisms [13].

## Conclusions

Rangeomorphs are typically envisaged to have been simple and passive organisms. However, *Hylaecullulus fordi* gen. et. sp. nov.—a multifoliate rangeomorph from the Ediacaran strata of Charnwood Forest (UK)—provides evidence for considerable architectural complexity and a truly modular organization, highlighting the importance of modularity in achieving large body size in phylogenetically disparate clades. Directed, enhanced growth in the form of eccentric branches illustrates their ability to respond to physical, external stimuli (such as damage) and conferred on them considerable environmental tolerance. Rangeomorph architecture was not immutable, and this plasticity has significant implications for the clade's systematic taxonomy. The presence of overcompensatory growth demonstrates that rangeomorphs were not passive bystanders in a dynamic environment, but were able to actively adapt and recover, putting to rest the notion of a tranquil Garden of Ediacara.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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### SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and two tables and can be found with this article online at https://doi.org/10.1016/j.cub.2018.08.036.

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### AUTHOR CONTRIBUTIONS

C.G.K. undertook the work and drafted the bulk of the manuscript, F.S.D. drafted sections of the Discussion section of the manuscript and Figure 4, and P.R.W. conceived the study and edited the manuscript.

### DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Deposited Data | | |
| Dynamic imagery (RTI) files of casts of fossil specimens | dynamic imagery (RTI) files of casts of the holotypes and paratypes are stored under the following DOI: https://doi.org/10.5285/d4aa9ec5-7cd4-4c35-aada-e7c4a119b64c | GSM106112; GSM106958; GSM106957; GSM106959; GSM106040; GSM106875; GSM106012; GSM106034 |
| Software and Algorithms | | |
| R software package | https://www.r-project.org/ | N/A |
| Other | | |
| Primary casts of fossil specimens | British Geological Survey, Keyworth, UK | GSM106112; GSM106958; GSM106957; GSM106959; GSM106040; GSM106875; GSM106012; GSM106034 |

### CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Charlotte Kenchington (cgk27@cam.ac.uk).

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

The *Caulerpa* algae were collected from Bristol Aquarium, and were cultured at 21°C in aerated open-system tanks, alongside other marine algae (*Galaxaura* and *Halimeda*), and sand anemones. Water salinity was 35 parts per thousand, and water pH was kept between 7.5 and 8.4. Nutrient addition was facilitated by addition of zooplankton every week, and nitrite and phosphate levels were tested every fortnight (using Salifert test kits). The algae were subject to diurnal cycles, with light provided by Aqua beam 1000 ultra HD marine lights

### METHOD DETAILS

#### Analysis of fossil specimens

The original fossil specimens remain *in situ* on the bedding plane, as they cannot be removed and are protected under UK SSSI legislation. Silicone rubber molds were taken from the bedding plane, and Jesmonite® resin casts produced from the molds. The casts form the material presented in this study.

Analysis of fossil specimens was conducted through detailed examination using a paleontological binocular microscope coupled with a directed light source (angle poise lamp). A camera lucida microscope and directed light source were used to make detailed line drawings of the fossils, which were then digitized in Adobe Illustrator. Measurements of specimen morphology were made with a ruler. High-resolution photographs were taken with a Canon EOS 7D Mark II and a Canon EOS 5D Mark III and were viewed through Adobe Photoshop.

#### Comparison to other known rangeomorphs

Rangeomorph taxonomy is currently in a state of flux [4, 41], but *Hylaecullulus* is readily distinguishable from all currently described taxa. It bears closest resemblance to *Bradgatia* Boynton and Ford [42] and *Primocandelabrum* Hofmann, O'Brien and King [43], both of which have a multifoliate construction and co-occur with *Hylaecullulus* on Bed B. However, *Bradgatia* lacks a stem and has a much smaller, bulb-shaped holdfast (Figure S4); its branching architecture is also distinct, being displayed, unfurled and radiating at all resolvable orders of branching (*cf*. [15]). While *Primocandelabrum* superficially resembles *Hylaecullulus* in its possession of a simple disc and a straight (albeit proportionally shorter) stem, its 'bushy' crown is notably triangular in preserved outline and its branches are coarser and arranged in a form resembling a candelabrum [43]. The poor preservation of the type specimens of *Primocandelbrum* from Newfoundland renders their finer branching architecture impossible to determine, but multivariate statistical analyses of specimens from Charnwood Forest consistently separates specimens of *Hylaecullulus* from *Primocandelabrum* ([4], their Figure 4). Two small multifoliate fronds formerly described as ''feather dusters'' have recently been described from Mistaken Point, Canada, and assigned to the taxon *Plumeropriscum hofmanni* [44]. While these specimens appear superficially similar to *Hylaecullulus* and

have been described as multifoliate, their primary branches appear to emanate along a central stalk ([44], their Figures 4 and 5(1)), they have smaller discs, proportionally much shorter stems, and a branching architecture that appears quite different to that of both *Hylaecullulus* and *Primocandelabrum* [41], but which remains to be fully described.

### Functional morphology of *Hylaecullulus fordi*

Based on its morphology and taphonomy, we interpret the living *H. fordi* organism to have had an open, bowl-shaped crown which was held aloft on a long, naked (i.e., not bearing branches), comparatively stiff stem, and was anchored to the shallow substrate by a large, oblate holdfast (Figure 1). As such, it represents an early example of the tall, arborescent form that was subsequently converged upon in the Phanerozoic by a diverse range of deep-water, sessile organisms, including pennatulaceans, crinoids and bryozoans (see [45]).

The crown of *H. fordi* was composed of equi-sized, partially-overlapping folia. There is no evidence to suggest that it was able to pivot or flex to any significant degree about its junction with the stem (as in stalked crinoids [46]), but each folium and primary branch was itself flexible. The net result was that a dense and near-continuous wall (both external and internal) of rangeomorph branches was presented to the water, enabling the crown to passively exploit currents from all directions equally. This made it particularly well-adapted to deep-water settings, where the direction and strength of benthic ambient flow may vary at any one location (e.g [47].).

Rangeomorph fronds are generally considered to be feeding structures [20, 21, 48], and their stems are argued to be a response to competition for vertically-distributed resources (i.e., tiering [49, 50]). The long, naked stem of *H. fordi* would seem to support this interpretation; it would have placed the organism's crown in a region of the water column with higher flow, thereby likely increasing the efficiency of exchange across its surface (*cf*. [51, 52]). However, the elevation of its crown overlaps with the fronds of most other taxa on the same bedding-plane surface, suggesting that it may have had an additional, or alternative, function to feeding. Rangeomorphs likely reproduced via waterborne propagules [53, 54], whose dispersal distance might be expected to increase with the height of the parent frond (*cf*. [55, 56]). Wide dispersal is particularly advantageous in disturbance-prone environments (e.g., [57]), such as the turbiditic settings occupied by *H. fordi* [28], and may have been the dominant driver of stem length in *H. fordi* and other frondose taxa with a naked stem.

## QUANTIFICATION AND STATISTICAL ANALYSIS

The R statistical package was used for simple statistical analysis involving regression of morphological proportions against one another (results detailed in the Systematic Paleontology section). The very low number of well-preserved specimens (n = 6) precluded further meaningful statistical analysis. Comparison of these fossil specimens with *Primocandelabrum* specimens was conducted using the R package FactoMineR [58, 59], and is detailed in [3].

## DATA AND SOFTWARE AVAILABILITY

Primary data is the casts housed at BGS Keyworth; dynamic imagery (RTI) files of casts of the holotypes and paratypes are stored under the following DOI: https://doi.org/10.5285/d4aa9ec5-7cd4-4c35-aada-e7c4a119b64c. R and the FactoMineR package are both open source [58, 59].

Access to the casts is controlled by the British Geological Survey, Nicker Hill, Keyworth, Nottingham NG12 5GG, UK.

# Viewing the Ediacaran biota as a failed experiment is unhelpful

Macroscopic organisms from the late Ediacaran period have often been described as failed experiments in the history of life. We argue that the field of Ediacaran palaeobiology should dispense with unhelpful historical classification schemes and embrace phylogenetic systematics if we are to establish the evolutionary relevance of these fossils.

Frances S. Dunn and Alexander G. Liu

The Ediacaran macrobiota — an assortment of macroscopic, largely soft-bodied organisms that lived during the ~30 Myr interval prior to the Cambrian period — have long been considered a palaeontological conundrum. Many fossils of these organisms exhibit unusual body plans that are unlike anything seen among living taxa (Fig. 1a–d) and it has proved difficult to resolve their relationships to extant groups. Individually and collectively, members of the Ediacaran macrobiota have been both allied with extant clades and deliberately set apart from them by suggestions that they were either 'failed experiments' in the history of life, or members of long-extinct higher-order clades[1]. Despite the role they have played in stimulating debate around these taxa, we argue that these latter viewpoints have hampered Ediacaran research. They have also created confusion within the wider community as to the placement of the Ediacaran macrobiota in the tree of life, forming a barrier to their incorporation within biological and developmental discussions. We advocate abandoning the failed experiment perspective and embracing phylogenetic thinking in order to make progress in determining the phylogenetic positions of these organisms and realizing their evolutionary significance.

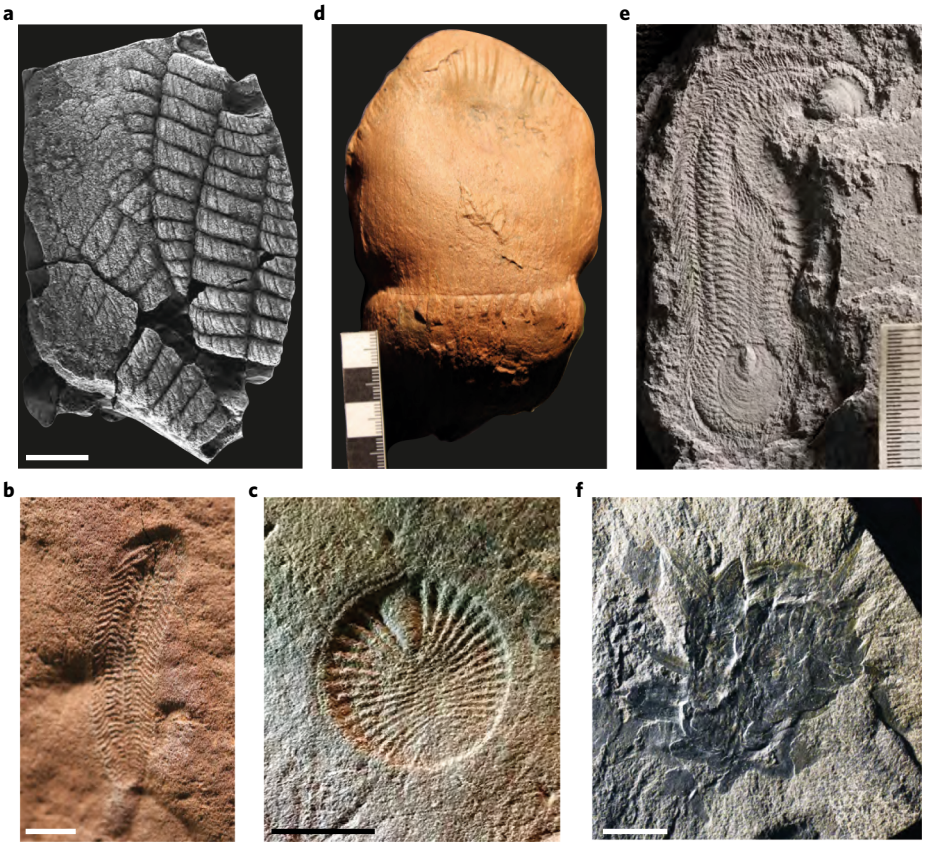


**Fig. 1 | Members of the Ediacaran macrobiota and Cambrian 'weird wonders'. a**, *Charnia masoni* from the White Sea region of Russia (PIN 3993–7018). **b**, *Spriggina floundersi* from South Australia (SAM P40137). **c**, *Dickinsonia costata* from South Australia (SAM P34224). **d**, *Ernietta plateauensis* from Namibia. All have been considered vendobionts[12], *Ernietta* was considered a petalonamid[5], and *Spriggina* and *Dickinsonia* were considered proarticulates[33]. **e**, *Halkieria evangelista* from Sirius Passet, Greenland. *H. evangelista* was described after publication of Gould's *Wonderful Life*[26], but has courted phylogenetic controversy. **f**, *Wiwaxia corrugata*. Scale bars in **a**, **b**, **c** and **f**, 1 cm. from the Burgess Shale (BRSUG 13384). Credit: D. Grazhdankin (**a**), M. Laflamme (**d**) and J. Vinther (**e**).

## Ediacaran taxonomic history

Unusual macroscopic impressions were described from bedding planes in what are now known to be Ediacaran-age strata from the 1840s onwards, but until the late 1950s it was held that such impressions could not represent Precambrian fossils. The prevailing wisdom suggested that all Precambrian rocks were 'Azoic', and pre-dated life, so the impressions were largely considered to represent either sedimentary structures or fossils within younger, Palaeozoic rocks. This situation changed following the seminal description of *Charnia masoni* from Charnwood Forest, United Kingdom, in 1958[2], and recognition that these fossils pre-dated the famous Cambrian Explosion and had a global distribution. Subsequently, the fossils began to be colloquially referred to as the Ediacara biota (later Ediacaran biota), after the Ediacara Hills locality in South Australia.

As taxa were formally described throughout the 1960s and 1970s, members of the Ediacaran biota were frequently considered to belong to derived animal

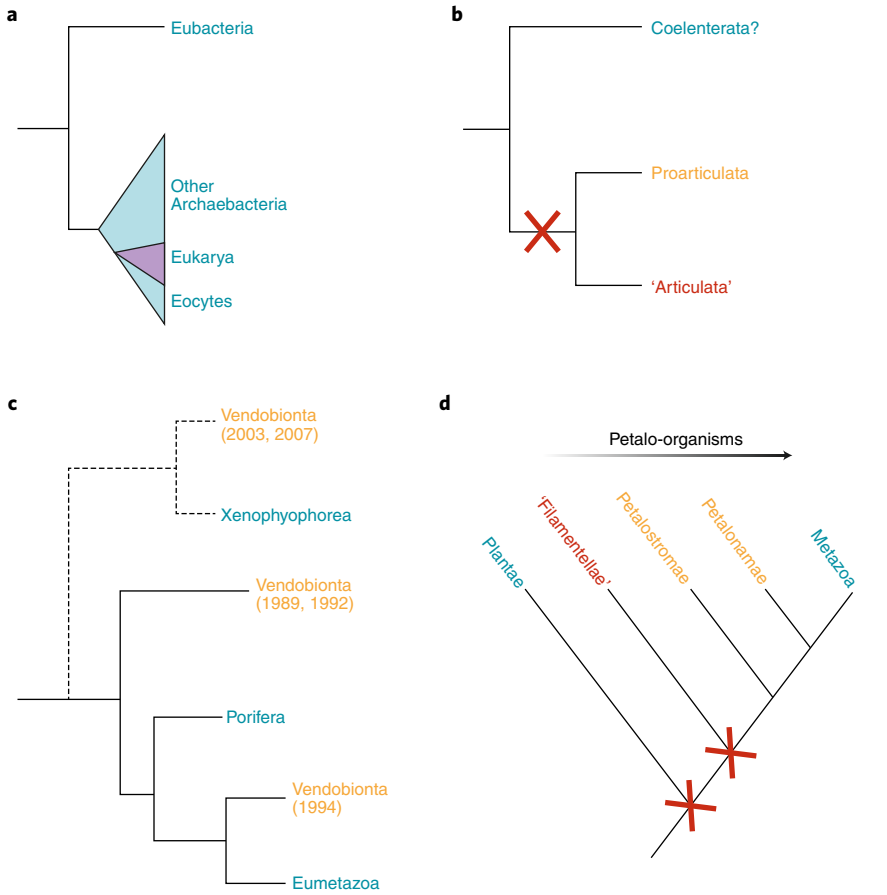


**Fig. 2 | How phylogenetic thinking affects our view of the Ediacaran macrobiota.** Crosses indicate nodes that are no longer supported, orange labels are proposed Ediacaran clades, red labels indicate clades that are now defunct. **a**, Molecular phylogenies predict a single origin of life, and therefore all organisms must fall within the known tree of life[34]. **b**, The Proarticulata (as originally defined[8,9]) cannot be reconciled with modern phylogenetic thinking, since the group to which it was most closely allied — the Articulata — has been rejected[10]. **c**, Previously hypothesized higher-order positions for the Vendobionta ultimately resolve them as either stem-group metazoans or eumetazoans[11–13], which remain viable phylogenetic hypotheses, or later as protists similar to xenophyophores[14,15] (dates indicated). **d**, The Petalo-organisms, as conceived by Pflug, represent a grade of organization between the animals and the plants, encompassing the Petalonamae and Petalostromae, which Pflug interpreted as clades[6]. Molecular phylogenetics has shown that the animal and plant kingdoms are not sister clades[35].

clades including the pennatulacean cnidarians[3] and the annelid worms[4], although non-metazoan opinions were occasionally expressed[2]. During the 1970s and 1980s, these hypotheses were challenged by a school of thought that sought to remove the Ediacaran biota from extant higher-order groupings and place them within new phyla or kingdoms. The phylum Petalonamae[5] ('Nama petals', named by Hans Pflug after fossils described from the Nama Group of Namibia) included several frondose taxa (for example, *Rangea*) and was initially considered to represent ancient animals, but was later revised to lie in a position intermediate to the animal and plant kingdoms, distinct from all living forms[6]. Pflug used the term Petalo-organisms to describe a grade of organization including the Petalonamae (with an anatomy closer to animals than plants) and Petalostromae (with an anatomy closer to plants than animals)[6] (Fig. 2d). A recent study has placed the phylum Petalonamae as sister to the Eumetazoa, but included a broader suite of taxa than were originally recognized by Pflug[7]. The phylum Proarticulata[8,9] included taxa such as *Dickinsonia* and was considered to comprise a clade of bilaterian animals with similarities to the (now defunct) superphylum Articulata[10]. Mikhail Fedonkin envisaged a situation in which truly segmented animals (Articulata) evolved from Ediacaran organisms with an offset form of bilateral symmetry, which themselves derived from radially symmetrical ancestors he considered related to jellyfish[8] (Fig. 2b). The radical Vendobiont hypothesis of Adolf Seilacher[11,12] united all Ediacaran taxa that displayed a 'serially quilted' anatomy (including those listed above) within the kingdom Vendobionta (meaning 'Vendian life' after the Russian stratigraphic term for the latest Precambrian), on the basis of taphonomic and constructional arguments. Seilacher and colleagues later modified these views, first revising the kingdom Vendobionta to an extinct sister phylum to the Eumetazoa[13], before considering vendobionts an extinct class[14] or subclass[15] of giant protists (Fig. 2c). This repeated revision may have been due to Seilacher's recognition that an extinct higher-order clade was an unsatisfactory solution[13,14] to the Ediacaran problem. Despite the radical nature of these hypotheses, they initially attracted considerable support, although questions were raised as to whether there was sufficient evidence to support the Vendobiont hypothesis, with alternative suggestions positing that the Ediacaran biota may simply represent a subset of Neoproterozoic biodiversity[16].

## Breaking up the Ediacaran biota

In challenging earlier metazoan interpretations, the hypotheses of the 1970s and 1980s brought the Ediacaran macrobiota to the attention of the wider scientific community, and laid the foundations for subsequent debate regarding their phylogenetic placement. A wealth of anatomical, palaeoecological and developmental specimen data has resulted from these investigations, and the majority of scientists now consider the Ediacaran macrobiota to be a polyphyletic grouping[17,18]. Some taxa are reasonably considered to be candidate metazoans, most notably *Dickinsonia*, whose metazoan placement is now supported by developmental, ichnological and biomarker studies[19–22], whereas other taxa are allied with non-metazoan eukaryotic groups, such as the protistan-grade *Palaeopascichnus*[23], or *Beltanelliformis* — interpreted as a cyanobacterial colony[24]. Today, as consensus tends away from a clade of the Ediacaran macrobiota, undermining the previously defined higher-order groupings (though with some exceptions, for example, ref. [7]), many researchers continue to cite Seilacher, Pflug and Fedonkin's works as a mechanism to demonstrate the idiosyncratic nature of these fossils. There is a particular tendency to cite only Seilacher's early work, asserting his idea that the Ediacaran macrobiota could represent an extinct kingdom as a viable hypothesis, while often neglecting

his later publications and taxonomic revisions, and overlooking the fact that he himself acknowledged the presence of metazoans (in the form of ichnofossils and macrofossils such as *Kimberella*) among Ediacaran fossil assemblages[11,14]. Several studies also continue to advance the view that the Ediacaran macrobiota are failed experiments by virtue of being extinct and not possessing known direct (living) descendants[1,25]. We argue that continued consideration of this viewpoint is detrimental to efforts to advance knowledge of Ediacaran macrobiota taxa.

## Weird wonders or extinct ancestors

Macrofossils from the Ediacaran period may well be strange, and many taxa remain difficult to interpret, but they can, and should, be interpreted within the framework of phylogenetic thinking[16]. Subjecting problematic fossils to hypotheses that displace them from extant groupings is not uncommon, as exemplified by the Cambrian organisms of the Burgess Shale (Fig. 1e,f). Stephen Jay Gould, among others, noted the unique anatomies of many fossils of the Burgess Shale, which he did not recognize in any extant animal phyla. Notably, rather than viewing these 'weird wonders' as failed experiments, Gould recognized the significance of the strange Cambrian taxa, some of which he did not consider to sit within any known animal phyla "of this or any former Earth"[26]. Furthermore, study of those unusual organisms has gradually resolved their relations to extant clades, as application of the stem and crown group concepts permeated invertebrate palaeontological research[27].

If we accept that life has a single origin (Fig. 2a), Ediacaran macrofossils must occupy a branch/branches in the known tree of life. Any phylogenetic framework must reflect contemporary knowledge, such that proposed Ediacaran clades are grounded in current phylogenetic consensus. For example, the Proarticulata were considered ancestors to the Articulata, a group that molecular data now suggest to be invalid[9], with metazoan segmentation evolving independently at least three times[28]. Only when the correct phylogenetic position(s) for the Ediacaran macrobiota has been established can their evolutionary success or failure be assessed (Fig. 2). Evolutionary success can be measured in many ways and does not necessarily correlate with survivorship: were trilobites an evolutionary failure? If Ediacaran taxa are resolved as being either paraphyletic, or a polyphyletic assemblage[17,18,29,30], it would be inappropriate to consider them a failed experiment, and in time we may consider some of the characters they possess (such as axial arrangement) as homologous with those of extant taxa. We recognize that at least some of the Ediacaran taxa were members of groups with extant representatives, including both metazoan (for example, *Dickinsonia*) and non-metazoan clades (for example, *Palaeopascichnus*). They persisted for 30 Myr, with evidence for considerable diversification, and they display the capacity to form complex ecosystems[31,32]. At their zenith, the Ediacaran macrobiota were arguably hugely successful, but we cannot rationalize any of these observations until the ultimate positions of the Ediacaran macrobiota in phylogeny are known.

## Moving forward

As the relatively young field of Ediacaran research continues to make rapid advances, we propose that Ediacaran macrofossil taxa should be considered on a case-by-case basis, with no underlying assumption of monophyly of the Ediacaran macrobiota. We advocate moving away from the use of leading terminology, which either deliberately divorces members of the Ediacaran macrobiota from living taxa, or asserts unproven relationships.

Assertion of whether or not the Ediacaran macrobiota represent failed experiments is premature while their phylogenetic positions remain unknown. Furthermore, if we are to fully appreciate the information these taxa can provide, we should define them not only by what sets them apart, but also by similarities to living forms. Future work detailing specific hypotheses of affinity should be based on positive evidence wherever possible, and be grounded in phylogenetic systematics, with careful consideration of a broad suite of characters, integrating anatomical, developmental and reproductive data, and recognizing the impact of different taphonomic regimes.

Holistic combination of such biological and palaeontological data offers our best route towards elucidating the early history of complex macroscopic organisms. The Ediacaran macrobiota must be restored to the known tree of life. ❐

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## Competing interests

The authors declare no competing interests.

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# ANATOMICAL AND ONTOGENETIC REASSESSMENT OF THE EDIACARAN FROND *ARBOREA ARBOREA* AND ITS PLACEMENT WITHIN TOTAL GROUP EUMETAZOA

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**Abstract:** Organisms in possession of a frondose body plan are amongst the oldest and most enigmatic members of the soft-bodied Ediacaran macrobiota. Appraisal of specimens from the late Ediacaran Ediacara Member of South Australia reveals that the frondose taxon *Arborea arborea* probably possessed a fluid-filled holdfast disc, the size and form of which could vary within populations. Mouldic preservation of internal anatomical features provides evidence for tissue differentiation, and for bundles of tubular structures within the stalk of the organism. These structures connect in a fascicled arrangement to individual lateral branches, before dividing further into individual units housed on those branches. The observed fascicled branching arrangement, which seemingly connects individual units to the main body of the organism, is consistent with a biologically modular construction for *Arborea*, and raises the possibility of a colonial organization. In conjunction with morphological characters previously recognized by other authors, including apical-basal and front-back differentiation, we propose that to the exclusion of all alternative known possibilities, *Arborea* can be resolved as a total group eumetazoan.

**Key words:** Ediacaran, Eumetazoa, frondose, modularity.

FOSSILS of macroscopic, soft-bodied organisms are found globally in late Ediacaran rocks of ~570–541 million years in age. These fossils are considered to document a polyphyletic assemblage of diverse and morphologically complex marine organisms (Fedonkin *et al.* 2007; Budd & Jensen 2017; though see Hoyal-Cuthill & Han 2018). The Flinders Ranges of South Australia (Dunn *et al.* 2019, fig. S1) offer an exceptional record of these taxa within fine to coarse-grained sandstones of the Ediacara Member of the Rawnsley Quartzite (Droser *et al.* 2019). This unit documents a variety of shallow-marine and deltaic depositional environments (Gehling 2000; Gehling & Droser 2013; Callow *et al.* 2013; Tarhan *et al.* 2017) and contains the impressions of thousands of organisms representing at least 30 distinct macrofossil taxa. Although the precise mechanism by which these fossils are preserved is a matter of considerable debate (Gehling 1999; Retallack 2007; Tarhan *et al.* 2016, 2018; Bobrovskiy *et al.* 2019; Liu 2019), there is a general consensus that Ediacara Member palaeoenvironments were reasonably high-energy marine settings, and that the seafloor upon which the organisms lived was covered by benthic microbial mat communities (Gehling & Droser 2009; Tarhan *et al.* 2017; Droser *et al.* 2019).

Fossil assemblages of the Ediacara Member are perhaps most widely known for possessing some of the oldest candidate bilaterian animals (Gold *et al.* 2015; Cunningham *et al.* 2017), including *Kimberella* (Gehling *et al.* 2014; Droser & Gehling 2015), *Parvancorina* (Paterson *et al.* 2017; Darroch *et al.* 2017; Coutts *et al.* 2017) and *Dickinsonia* (Evans *et al.* 2017; Hoekzema *et al.* 2017; Bobrovskiy *et al.* 2018; though see Sperling & Vinther 2010). Alongside these taxa, frondose organisms (Glaessner 1971) assigned to the unranked morphogroups Rangeomorpha and Arboreomorpha (Erwin *et al.* 2011) represent a comparatively little-studied component of the Australian Ediacaran assemblages. Frondose taxa are more typically known from older, deep-marine Ediacaran palaeoenvironments in Newfoundland (Canada) and England (Liu *et al.* 2015), but in the Ediacara Member they occur in shallow-marine facies interpreted to reflect deposition in delta front, sheet-flow and mass-flow


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depositional environments (Gehling & Droser 2013; see also Tarhan *et al.* 2016). Frondose taxa represented in the Ediacara Member include *Charnia* (Gehling & Droser 2013), *Bradgatia* sp. (Droser & Gehling 2015) and *Pambikalbae* (Jenkins & Nedin 2007), and their facies distributions contrast with the shoreface and wave-base sand settings in which non-frondose taxa are most abundant (Gehling & Droser 2013). However, numerous discoidal impressions, initially interpreted as medusoids (Glaessner 1984) but more recently reinterpreted as holdfast structures of frondose organisms (Tarhan *et al.* 2015), may indicate that frondose taxa were reasonably abundant within all Ediacara Member palaeoenvironments. Taphonomic variation in disc expression currently precludes identification of original taxa in situations where the frond is absent (Gehling *et al.* 2000; Burzynski & Narbonne 2015; Tarhan *et al.* 2015).

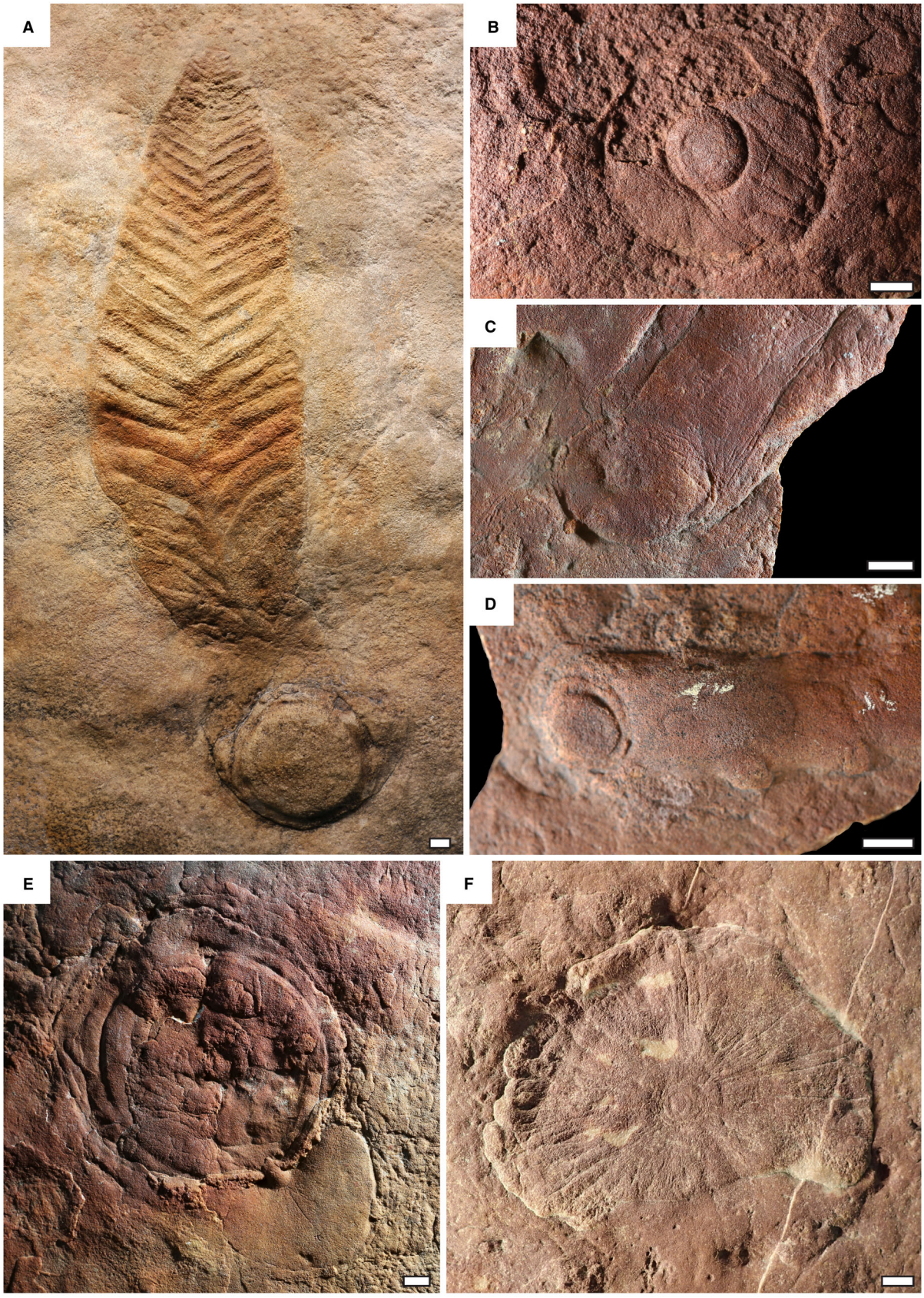
The most common frondose taxon in the Ediacara Member is *Arborea arborea* (Glaessner & Daily 1959), the organism after which the morphogroup Arboreomorpha is named (Laflamme & Narbonne 2008; Erwin *et al.* 2011; Laflamme *et al.* 2018). *Arborea arborea* can be abundant on individual bedding surfaces within wave-base, sheet-flow and mass-flow facies (Laflamme *et al.* 2018; see *Charniodiscus* in Gehling & Droser 2013), and also occurs in low densities alongside more typical components of the Ediacaran biota (Coutts *et al.* 2016). Some *Arborea* specimens may have exceeded lengths of two metres (Dunn *et al.* 2019, fig. S2), making this one of the largest known Ediacaran macro-organisms. A detailed reassessment of frondose taxa in South Australia synonymized specimens previously assigned to *Charniodiscus oppositus*, *Charniodiscus arboreus*, *Rangea arborea*, *A. arborea*, and even some *Charnia* sp. within *A. arborea*, following determination of the three-dimensional structure of *Arborea* branches (Laflamme *et al.* 2018). That study diagnosed *Arborea* as a bifoliate frond with second order branches that lack rangeomorph sub-divisions (consistent with Laflamme & Narbonne 2008; Erwin *et al.* 2011; Brasier *et al.* 2012; Laflamme *et al.* 2018): an arrangement that is distinct from that observed in the type *Charniodiscus* material from the UK. We concur with these opinions, but to avoid confusion we resist drawing morphological comparison to arboreomorph taxa described from outside of Australia in this study. Whereas rangeomorph taxa have historically been assigned to multiple, often contradictory, phylogenetic positions within the eukaryotes (summarized in Dunn *et al.* 2018), *Arborea* has only seriously been proposed to fall within either the hypothetical phyla Petalonamae (Pflug 1970, 1972; Hoyal-Cuthill & Han 2018) or Vendobionta (formerly Kingdom Vendozoa, more recently considered to be a class or order of rhizoid protists; Seilacher 1989, 2007; Buss & Seilacher 1994; Seilacher *et al.* 2003), or the Cnidaria (Jenkins & Gehling 1978). We here reassess the morphology of multiple *Arborea* specimens from South Australia, and build upon recent studies (Laflamme *et al.* 2018) to propose a new model for *Arborea* anatomy.

## METHOD

We assessed 56 specimens that have either been historically assigned to *Arborea*, or recently synonymized with that taxon (Laflamme *et al.* 2018), in the collections of the South Australia Museum (SAM; Figs 1–5). Specimens were collected from South Australian fossil localities within the Ediacara Member of the Rawnsley Quartzite between 1957 and 2015; namely the Ediacara Conservation Park, the Flinders Ranges National Park, and National Heritage Site Nilpena (Dunn *et al.* 2019, fig. S1). Many of the studied specimens are incomplete, and when originally catalogued by their discoverers (who include M. Wade, M. Glaessner, W. Sun, R. Jenkins and J. Gehling), they were assigned to several different taxa. We follow recent synonymization (Laflamme *et al.* 2018) of these specimens, but note that we cannot categorically reject the possibility that some specimens may derive from a different taxon. Care has been taken to base the principal findings of this study only on specimens we are confident derive from a single taxon conforming to the most recent diagnosis of *A. arborea* (Laflamme *et al.* 2018).

Most of the studied specimens are preserved as positive hyporelief impressions on the bases of sandstone beds, but some reflect composite impressions of original external as well as internal anatomy. A small number of specimens are preserved in three dimensions, as sand-filled casts typically documenting external morphology (Laflamme *et al.* 2018), while one new surface (from Nilpena; Dunn *et al.* 2019, fig. S2) possesses very large specimens preserved in positive epirelief. These latter specimens remain *in situ* in the field. Key anatomical findings of Laflamme *et al.* (2018) include evidence for 'dorso-ventral' differentiation in *Arborea*, the inferred

**FIG. 1.** *Arborea arborea*, showing variability in the size and shape of *Arborea* holdfasts. All figured specimens are preserved as positive hyporelief impressions. A, complete specimen SAM P19690a, with an articulated holdfast. B, SAM P12888, with a single central boss and a stem whose width < holdfast diameter (stem is at bottom right). C, SAM P40332, holdfast with a stem with width = holdfast diameter. D, unlabelled specimen '52', holdfast with a stem of width ≥ holdfast diameter. E, large holdfast, seemingly showing a fan of sediment (bottom right) emerging from the holdfast interior, SAM P40309. F, holdfast of a large frond (SAM P49366), with radially arranged striations. All scale bars represent 10 mm. Colour online.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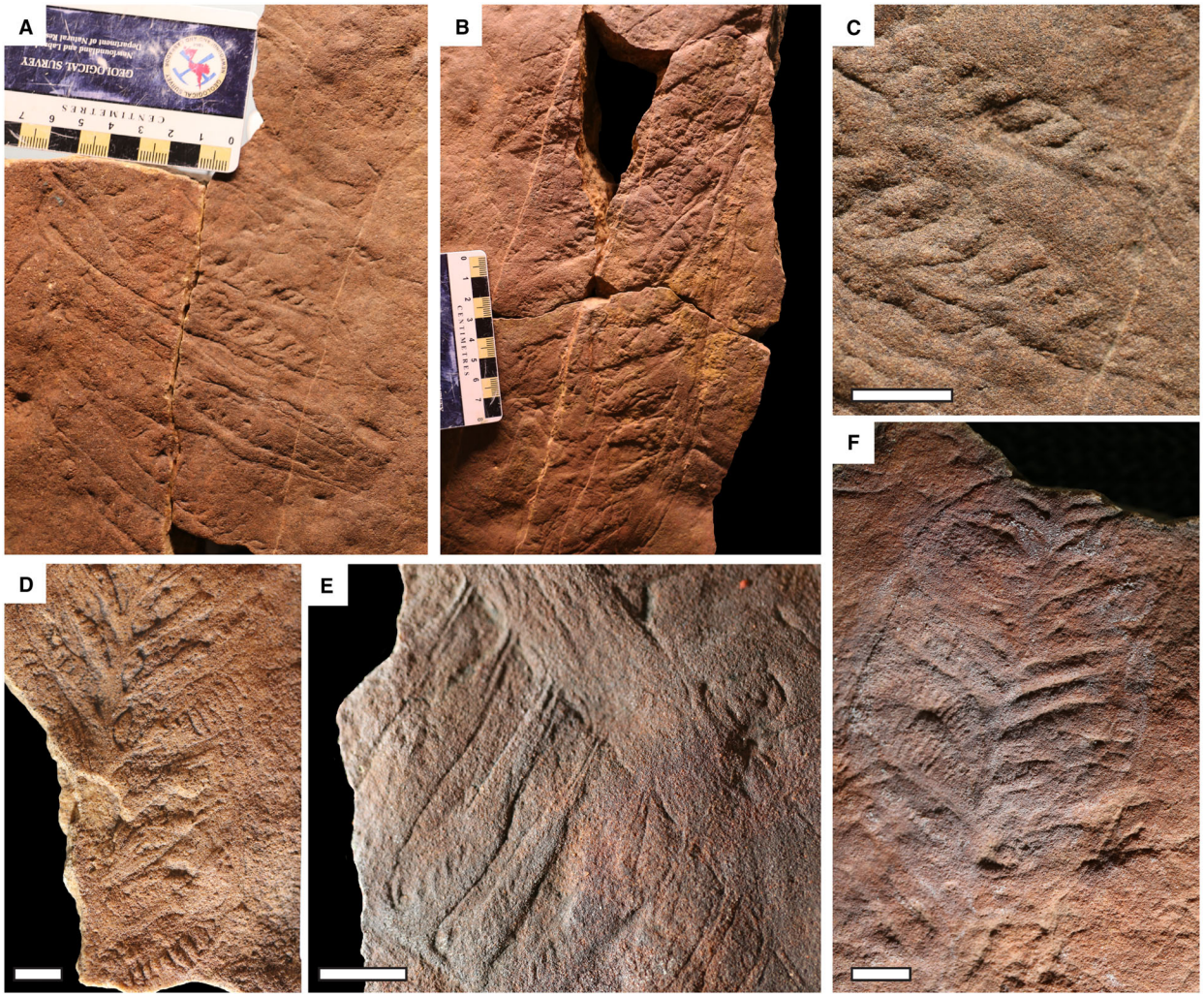


**FIG. 2.** Detailed lateral branch morphology in *Arborea* specimens demonstrating 'pod' and unit anatomy. A–C, SAM P40858, with lower order branches pointing upwards in A, but downwards in B on the opposite side of the frond, demonstrating that in life, these units were free to pivot along the branch axis; C, close up of lateral branches in A, with individual units showing comb-like sub-divisions. D, SAM P40952, lateral branches exhibiting units in the absence of 'pods'. E, SAM P42686, showing the connection between the 'pod' and the wide central stalk. F, SAM P40775, with units arranged on branches either side of a narrow stalk. All scale bars represent 10 mm. Colour online.

preservation of internal structures, and the ability for sediment to become incorporated within the specimens. We confirm those findings but interpret several additional anatomical observations to be biologically informative. We refrain from using phylogenetically loaded terminology in our description of *Arborea*, for reasons discussed in previous publications (Dunn *et al.* 2018).

## RESULTS

*Arborea arborea* is composed of a holdfast, a stem, and an ovate, leaf-like frond comprising two rows of lateral branches (following Runnegar 1995) emanating from either side of a central stalk (Fig. 1A). Each branch within the frond comprises smaller sub-divisions (here called units, previously referred to as second order branches) that appear to lie behind a covering structure, or 'pod' (*sensu* Laflamme & Narbonne 2008; Fig. 2). Known *Arborea* specimens range in size from complete specimens of just a few centimetres in length to incomplete fronds of over one metre (Dunn *et al.* 2019, fig. S2). The smallest studied specimen (SAM P40785; Fig. 3A) possesses ~19 lateral branches per row and is 3.5 cm in length, whereas specimens longer than ~4.5 cm in length (SAM P48727, Fig. 3E; or P19690a, Fig. 1A) often possess >30 lateral branches. One large incomplete frond possesses at

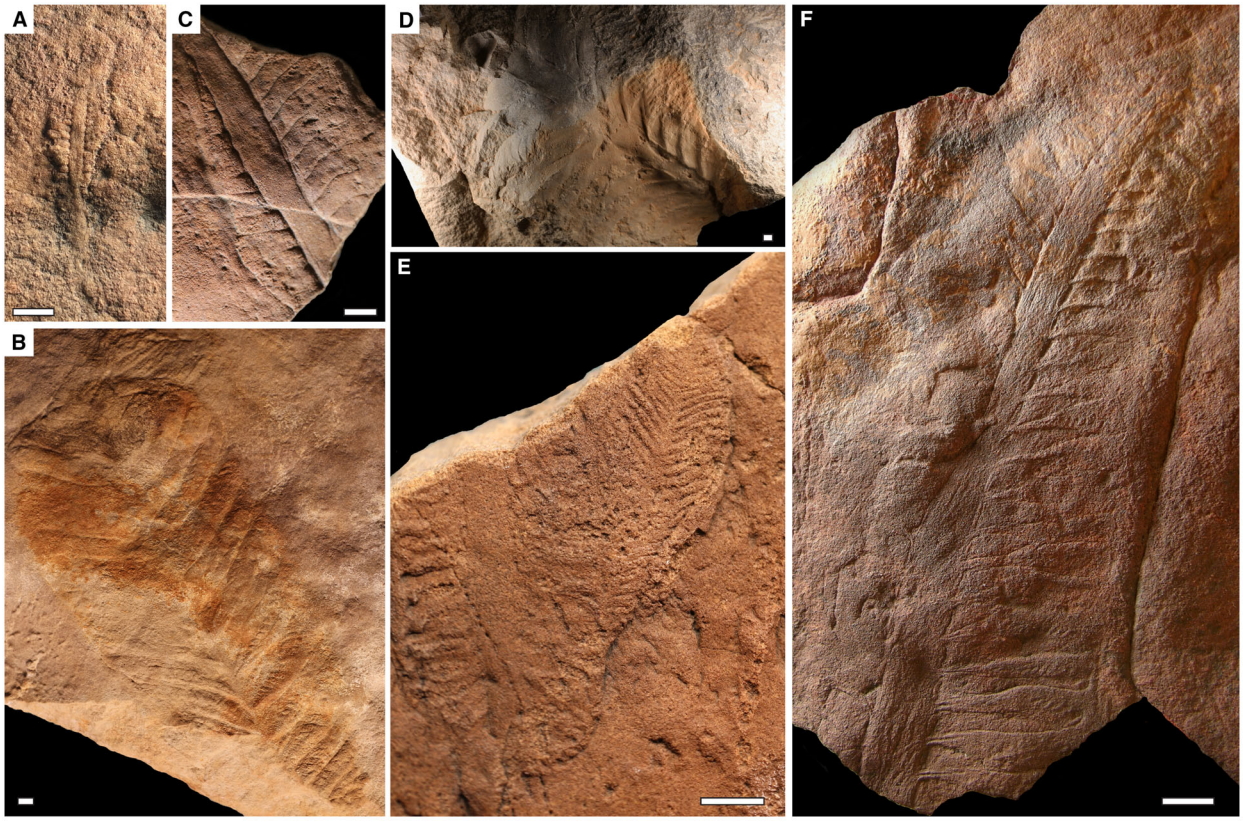


**FIG. 3.** The 'sidedness' of *Arborea*. A, SAM P40785, the smallest specimen studied, with no visible sub-division of lateral branches. B, SAM P19690b, the tip of the frond is over-folded revealing the two sides of the organism; the bottom of the frond shows 'pods' and units, and the tip of the frond (over-folded section) shows undifferentiated rectangular branches with no visible 'pods' or units. C–D, SAM P34499 and SAM P35704b respectively, exhibiting smooth rectangular panels interpreted as the 'back' of the organism. E, SAM P48727 with lateral branches visible in one of the smallest described specimens. F, SAM P42686, 'pods' and units clearly visible (interpreted as the 'front' of the organism), with rectangular undifferentiated branches absent. All scale bars represent 10 mm. Colour online.

least 33 branches (SAM P40858), while a newly discovered specimen has >49 (Dunn *et al.* 2019, fig. S2). The frond outline transitions from tapering (in terms of branch length) at both tips in smaller specimens (fusiform), to tapering primarily at the apical tip. In a specimen ~4.5 cm in length (Fig. 3E) the basal-most branches are ~40% of the length of the longest branches, whereas in a specimen ~30 cm in length (Fig. 1A) the basal-most branches are ~78% of the length of the longest branch. The following description provides a model of the anatomy of *Arborea* (Fig. 6).

*Arborea* possesses a holdfast structure that may variously exhibit a small number of concentric rings (Fig. 1A, D), a prominent but smooth central boss (Fig. 1B; Dunn *et al.* 2019, fig. S3), or multiple radial grooves (Fig. 1F). Such structures have, when found in isolation, previously been referred to discoidal taxa such as *Aspidella* or *Eoporpita* (Wade 1972; Tarhan *et al.* 2017), but those are now largely interpreted as organ taxa, with much of the observed variation in discoid morphology asserted to be taphonomic in origin (Tarhan *et al.* 2015; Burzynski *et al.* 2017). The holdfast connects at its centre to a single stem (Fig. 1), and varies in size relative to the width of the stem within the studied population, being of roughly equal diameter in some specimens (Fig. 1C, D), or 3–4 times larger in others (Fig. 1F). This variation does not appear to be directly correlated to specimen size (here measured as frond length), with a specimen of ~30 cm in length (SAM P19690a; Fig. 1A) possessing a holdfast of 108.6 mm diameter, while another ≫74.45 cm (SAM P40858) possesses a holdfast of only 82.2 mm diameter. In one specimen, a holdfast is associated with an arcuate fan of sandy material (Fig. 1E). This fan does not exhibit any of the morphological characters typical of frond holdfasts (e.g. a central boss, or radiating striations), and a narrow projection of sand associated with the holdfast margin appears to connect the base of this disc to the 'arcuate fan' that lies stratigraphically above it. This relationship would be highly unusual in two overlapping discs. Together with its distinct morphology, this leads us to

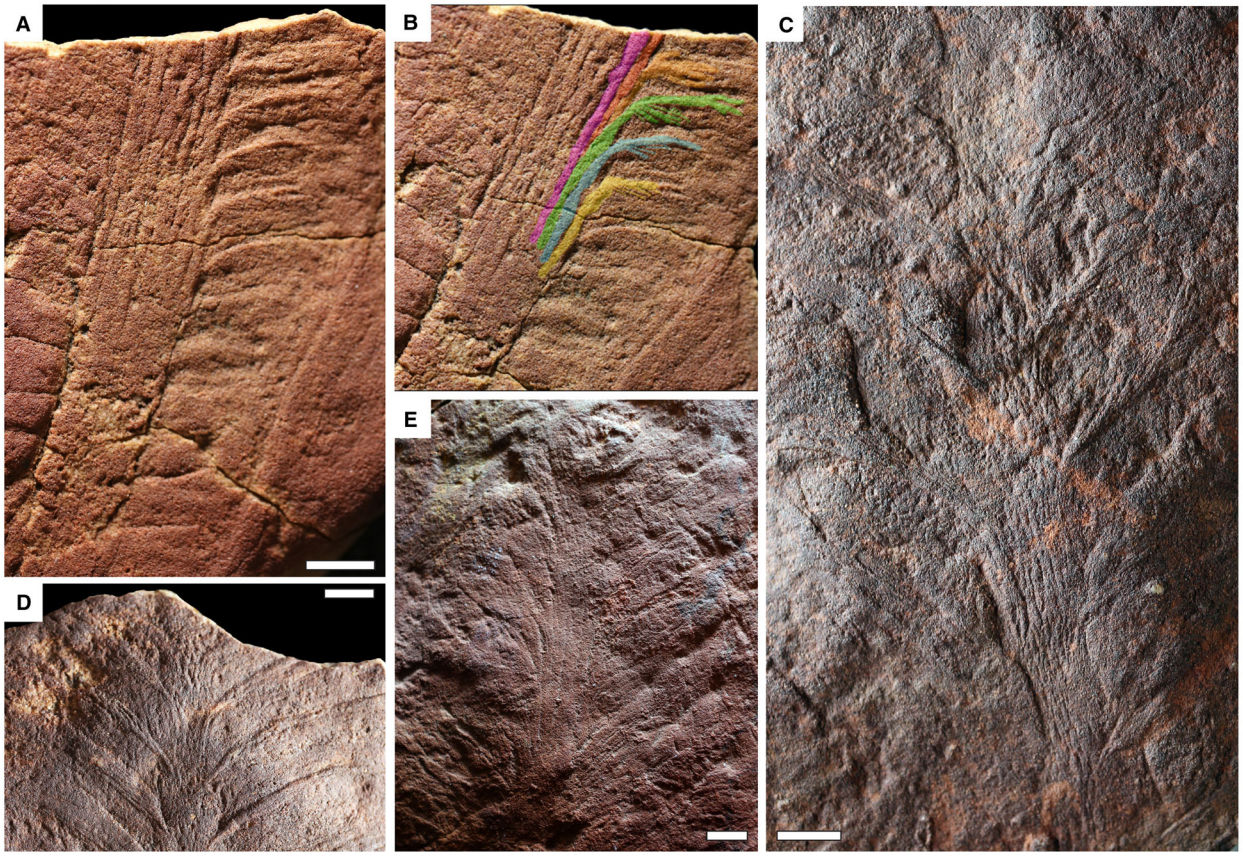


**FIG. 4.** The fascicled arrangement of branches in the stem of *Arborea arborea*. A–B, SAM P47800, individual tubular structures in the stem; A, tubular structures connecting in a one-for-one relationship to individual lateral branches, highlighted in B. These branches then either de-bundle or branch within the individual lateral branch. C–E, SAM P13801, SAM P47799 and SAM P51200 respectively, exhibiting the fascicled arrangement of tubular structures running up the stem and into individual lateral branches, where they divide further. All scale bars represent 10 mm.

postulate that this fan does not reflect the impression of a second holdfast. We instead suggest that the sediment fan represents fluidized sediment emanating from a break in the wall of the large holdfast. The sediment fan is similar in morphology to lobate structures produced by fluid escape in other late Ediacaran mat-bound sedimentary units (e.g. the Longmyndian Supergroup of the UK; Menon *et al.* 2016).

Within the studied population, the stems can exhibit variable relative lengths (see Fig. 1A for a very short example), an observation that in other taxa has been considered functionally significant in terms of ecological tiering (Laflamme *et al.* 2012) or reproduction (Mitchell & Kenchington 2018). Stem length shows no clear relationship to frond size. Stems can be smooth and featureless (Fig. 1D), finely wrinkled (Fig. 1C) or composed of numerous grooves and ridges that run parallel to their length into the stalk (Figs 3F, 4). These structures distally taper in width, and do not branch or amalgamate within the stalk. They do not continue into the holdfast in any studied specimen, and appear to record tubular structures extending up the stalk (Fig. 4). Along the length of the frond, individual tubes successively exit the stalk and become the primary axis for individual lateral branches (e.g. Fig. 4A). The tubes can connect to branches either at the margin of the stalk (Figs 2E, 4A, C), or closer to its centre (Fig. 4D).

The frond itself is composed of two rows of lateral branches (one on either side of the central stalk; Laflamme *et al.* 2018), which appear either bilaterally or alternately arranged across the midline. The longest branches are present in the middle of the frond, with branch lengths diminishing both apically and basally (Fig. 1A). *Arborea* has previously been described as possessing branches resembling 'pea pods' (Laflamme *et al.* 2018), with two sheet-like structures representing a continuation of the stalk wrapping up and around the serially-arranged units. Observed fronds typically show one of two possible branch variants. The first comprises solid, almost featureless rectangular blocks, which can

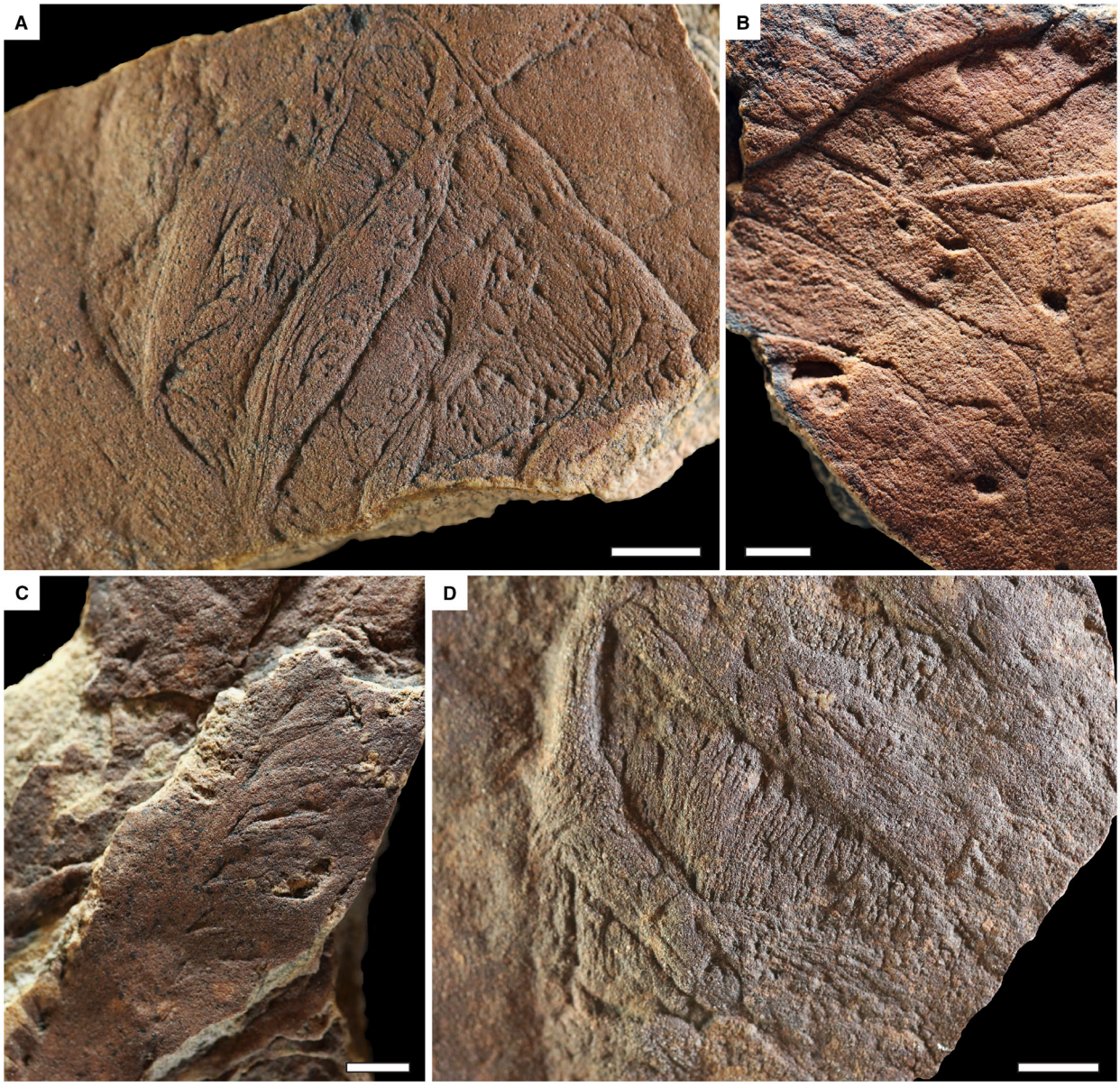


**FIG. 5.** The backing sheet and lateral margin of *Arborea*. A, SAM P40786, with lateral branches splitting off the stalk (at left), but also connecting to the lateral margin; linear striations running apico-basally between and seemingly beneath the lateral branches may indicate the presence of a wrinkled backing sheet underlying the branches. B, SAM P40772, exhibiting a striated surface, interpreted as the backing sheet, in between the lateral branches. C, SAM P40369, individual branches connecting to a lateral margin (at right). D, SAM P40773, revealing a striated backing sheet between the relatively smooth lateral branches. All scale bars represent 10 mm. Colour online.

occasionally exhibit transverse linear ornament. These abut one another to form a continuous smooth impression (e.g. Figs 1A, 3C). The second variant exhibits branches with a lenticular 'pod', partially covering a row of finely divided units along the length of the lateral branch (Fig. 2). In such cases, each lateral branch attaches to the central stalk via a single tubular structure (e.g. Figs 2D–E, 4). The distal end of each branch can also attach to the frond margin in some specimens, along what has previously been termed an undivided or marginal rim (Glaessner & Daily 1959; Jenkins & Gehling 1978). The secondary units within individual lateral branches can be oriented either apically or basally even within individual specimens (compare Fig. 2A, B), suggesting that they could pivot along the branch axis. In the smallest specimens, lateral branches appear bulbous, with no units visible (Fig. 3A). Each unit is rectangular to tear-shaped and may exhibit one order of transverse sub-

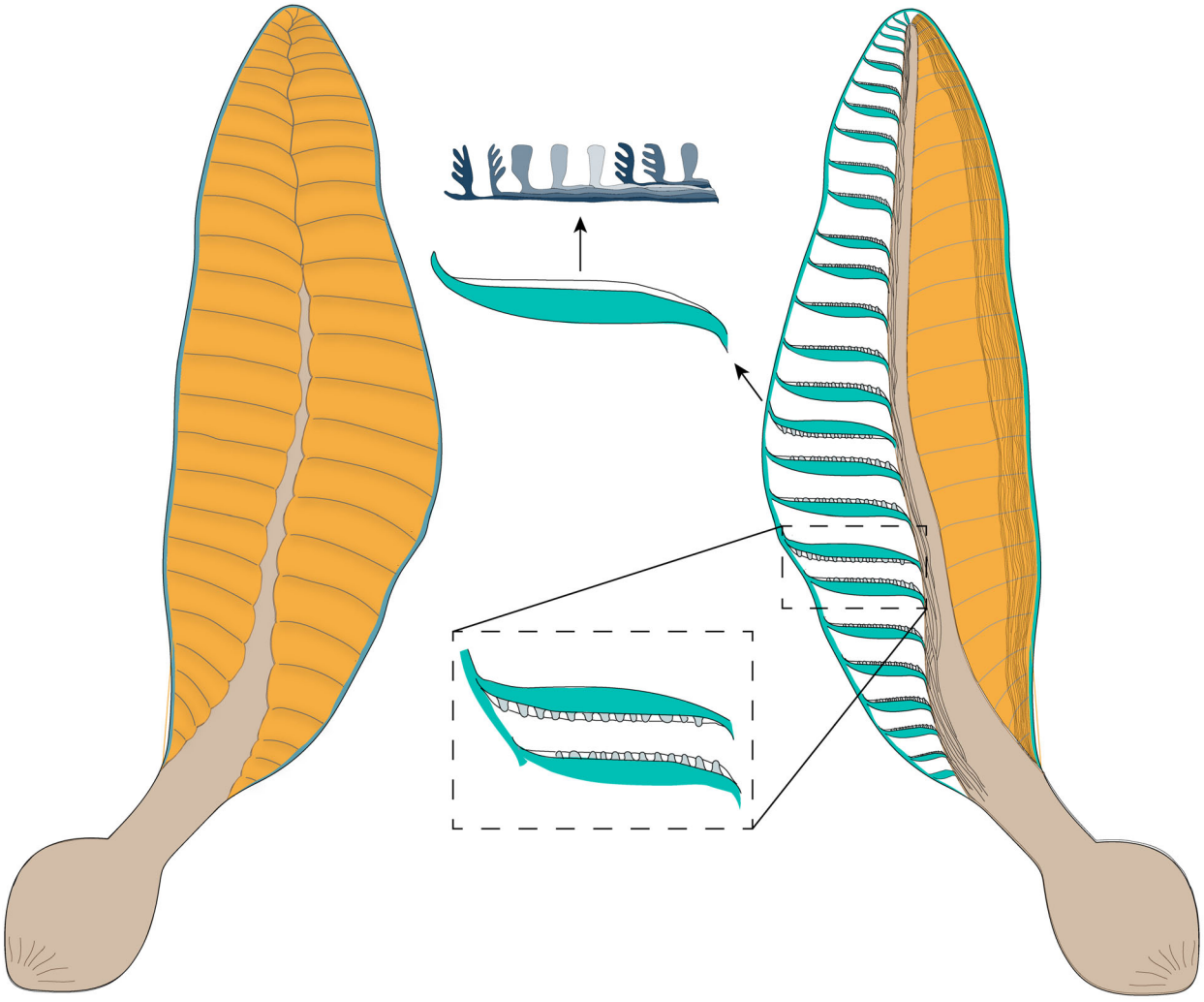

**FIG. 6.** An anatomical reconstruction of the Ediacaran frondose taxon *Arborea arborea*, based on the features discussed in this study. The 'back' (left) and 'front' (right) faces of the organism are shown. The right-hand side of the front shows the organism with the 'pods' and units (i.e. the branches) removed to reveal the underlying backing sheet. Inset: fine-scale arrangement of units within the 'pod'. Units are each connected to their own tubular, stolon-like structure running into the stalk. Note that pods (green) are free to pivot about the lateral branch axis.

divisions along its length (Figs 2A, 3B; termed striations by Hoyal-Cuthill & Han 2018). These subdivisions appear to emanate in a single direction, suggesting a comb-like morphology for individual units.

The tubular structures running along the stalk connect to individual lateral branches in a one-to-one, fascicled, arrangement (Fig. 4). They then divide and orient themselves perpendicular to the lateral branch, before branching further, or debundling, at regular intervals (Fig. 4A–C). Specimens only rarely exhibit both tubular structures and branch units. The tubular structures run up the lateral branches to their distal margin, dividing/debundling as they go to correspond, in a one-for-one relationship, with the expected positions of individual units that sit within the 'pod' (Figs 4A, C; 5A).

The lateral branches may additionally be underlain by a set of unidirectional linear striations arranged parallel (e.g. Fig. 5A, D) or oblique (Fig. 5B) to the marginal rim. These can be present across the entire width of the frond between the stalk and the lateral margin. This striated fabric may reflect a continuous sheet-like structure.

## DISCUSSION

### *Model of anatomy*

Holdfasts are rarely preserved in association with complete *Arborea* fronds, most likely due to both the large size of *Arborea* specimens and because in life much of

the holdfast may have been located beneath the sediment–water interface, and thus in a different plane of preservation (although preservation varies between beds; see Fig. 1A and Dunn *et al.* (2019, fig. S3) for examples of fronds and holdfasts preserved in the same plane). In the three clearest examples within the studied collection, where the complete frond and holdfast disc are articulated, there is no relationship between the size of the frond and the size of its associated holdfast, although the smallest specimen does possess the smallest holdfast structure. Laflamme *et al.* (2018) referred to one specimen (their fig. 2.2) as 'deflated'. Our observation of variable holdfast size is consistent with this interpretation. The ability of holdfasts to deflate, either during life or upon burial, is consistent with the organism being able to control and modify its shape. This interpretation is supported by the specimen with a fan of what appears to be escaping sediment (Fig. 1E), which may imply fluid fill within such holdfasts, and thus a potential ability to hydrostatically control holdfast size. An ability to actively modulate holdfast shape and size would imply the presence of contractile (muscular?) tissue (Jenkins & Gehling 1978), though in the absence of further data, contraction due to dehydration could represent an alternative possibility. An absence of contraction rims or disturbed sediment surrounding the specimens may suggest that this is unlikely.

The stalk of *Arborea* was likely to have originally been cylindrical (Laflamme *et al.* 2018), as supported by observed variation in the position of branch connection points, and the presence of both alternating and bilaterally symmetrical branch arrangements amongst the studied population. We consider at least some of this variation to result from rotation of the branch connection points out of the plane of preservation prior to compression of the cylindrical stalk, followed by their composite moulding on to the stalk in their 'rotated' positions. It is difficult to determine whether lateral branches were originally arranged in an alternating or bilaterally symmetrical manner, since these two branching arrangements are observed in almost equal numbers within the studied population.

The fascicled arrangement of tubular structures in the stalk and within the lateral branches (Fig. 4) appears to document the connection of individual units along each branch to the central part of the organism. These tubular structures extend into the stalk beyond the position expected of branches, and since *Arborea* is only known to possess two rows of branches, we do not consider the tubes to represent overprints of other lateral branches. The consistent one-for-one relationship of the tubes with individual lateral branches in multiple specimens precludes taphonomic interpretations such as wrinkling of an epithelium or a similar soft-tissue structure. It is not currently possible to determine whether these tubes were originally hollow or solid structures.

Since the tubular structures are most commonly observed when the pods and units assumed to reflect the exterior surface of the lateral branches are not preserved, we interpret the tubes as internal anatomical features. The relatively sharp boundary between these tubular structures and the smooth stem in some specimens (e.g. Fig. 4A) indicates that this difference is unlikely to be taphonomic in origin. Differential preservation of the smooth exterior of the stalk and these internal structures (Figs 3C; 4A, C) implies that they originally comprised different anatomical structures, suggestive of 'tissue' differentiation.

The tubular structures we report were documented and termed spicules by Glaessner & Wade (1966; see also Jenkins & Gehling 1978), an interpretation focusing on their sharp outlines and straight trajectories. However, their preservation as impressions rather than as biomineralized structures, the observation that they bend to extend into the branches, the presence of examples that curve and are clearly not straight within the stalk, and their ability to divide within the lateral branches (Fig. 4), lead us to question this hypothesis. True spicules in extant poriferans and cnidarians exhibit a variety of form. In cnidarians, calcitic spicules represent a derived condition, being present only in the Octocorallia. They are secreted by the mesoglea and are largely concentrated in the base of the colony, but may also be present in polyp leaves, or on anthocodia (Hyman 1940). In siliceous sponges, spicules are generally classified as either microscleres (smaller 'flesh' spicules) or megascleres (the main skeletal support elements). Megascleres are known to reach sizes of up to 3 m (and be up to 8.5 mm in diameter) in the basalia of *Monorhapis chuni*, where they function as a stalk (Müller *et al.* 2007). More commonly, microscleres are on the order of 1–60 μm, whereas megascleres are between 60–200 μm, and both can bundle and inter-weave (e.g. in the order Halichondrida; Hooper & van Soest 2004). The continuation of tubular structures up the stalk of *Arborea* and into its individual branches and units is an arrangement not seen in any extant spicular organism.

An alternative possibility, favoured here, is that the tubular structures in *A. arborea* represent non-mineralizing, stolon-like projections, consistent with their length, seemingly flexible nature, and one-to-one relationship with individual lateral branches and then units (Fig. 4). Stolons or stolon-like projections represent a derived condition in the Bilateria, but are nevertheless possessed by several invertebrate groups (e.g. the Bryozoa (Osborne 1984) and Entoprocta (Nielsen 2012, p. 201)) as well as many plants (de Kroons & Hutchings 1995) and algae (Ceccherelli *et al.* 2002), while fungal mycelia (Benjamin & Hesseltine 1949) may also produce thread-like projections. Horizontal creeping stolons are known in many land

plants (e.g. *Fragaria ananassa*; Savini *et al.* 2008) and in algae (e.g. *Caulerpa prolifera*; Ceccherelli *et al.* 2002). In the siliceous and calcareous sponges, stolons can take a variety of forms, including creeping stolons (e.g. the calcareous sponge *Leucosolenia*; Padua & Klautau 2016) and reinforced structural stolons (e.g. the carnivorous demosponge *Chondrocladia lyra*; Lee *et al.* 2012). Poriferan stolons are not known to bundle. Cnidarian clades exhibit stolons with morphological expressions that encompass horizontal creepers, and (particularly in the Hydrozoa) bundled vertical projections (Schuchert 2001), or fascicles. These fascicles may surround a 'true' stem but be encompassed by periderm (e.g. in the hydrozoan *Plumularia*; Hyman 1940 fig. 116) or may themselves comprise the stem (e.g. in the hydrozoan *Eudendrium*; Hyman 1940, fig. 116). Such fascicled branches provide the most similar extant analogue for the arrangement of tubular structures seen in *A. arborea*.

If the holdfast of *Arborea* was hydrostatically regulated, some form of hydraulic system would be expected. We find no firm evidence for any such system, but note that some extant hydraulic systems, such as the inhalant and exhalent siphonozooids of pennatulaceans (Williams *et al.* 2012) are unlikely to be expressed in known specimens of Ediacaran frondose taxa due to their position beneath branch attachment points along the stalk. Alternatively, the fascicled tubes may have been involved in hydraulic regulation, particularly if the individual units to which they connect were open to the water column.

The 'backing sheath' in *Arborea* (the apparent connective structure that joins the stalk with the marginal rim) may have anchored the lateral branches in place, though Laflamme *et al.* (2018) proposed that the rim could alternatively reflect folding of the distal tips of the lateral branches. The Russian frondose taxon *Charniodiscus yorgensis* has also been interpreted as having first-order branches that are constrained along their horizontal axes, but unlike *A. arborea*, *C. yorgensis* is reconstructed as exhibiting full branching units on both sides of the organism (Ivantsov 2016). No fascicled branching arrangement has been noted in *C. yorgensis* despite the pyritization of internal anatomical features.

The observation that 'pods' and units can be present or absent in *Arborea* specimens, even within individual specimens (Fig. 3B), is consistent with the suggestion that they are only present on one side of the organism, conferring front–back differentiation (Fig. 6; Jenkins & Gehling 1978; Laflamme *et al.* 2018). The 'back' of the organism comprises the backing sheath, subdivided into rectangular blocks defined by lateral seams. The linear striations observed running behind lateral branches in certain specimens (e.g. Fig. 5A) are interpreted to reflect either the inner surface of the backing sheath, or a distinct layer within the organism. In addition to the clear apico-basal differentiation of the organism, this character could potentially assist in constraining phylogenetic affinities.

Lateral branches were attached to the stalk by both a tubular continuation of external tissue, and by the internal tubular projections (leading to apparent pairing of connections in some specimens; Gehling 1991). Lateral branches consist of two main elements: the 'pod', which was constructed of two lens-shaped sheets (not bound to each other at either their apical or basal margins) and the sub-rounded to comb-shaped units (Fig. 6, inset), which lay within the pod. Previous studies have considered subdivisions within second order units to reflect wrinkling of a soft tissue structure (Laflamme *et al.* 2018) but their consistent morphology both within and across specimens leads us to consider them biological features. We note that the first order branches of *Arborea*, being comprised of a lenticular 'pod' and subdivided units housed therein, differ fundamentally in architecture from the linear subdivisions seen in second and third order units. This distinction does not fit the 'self-similar' branching definition of the Rangeomorpha, and we therefore follow previous workers (e.g. Laflamme & Narbonne 2008) in considering branching arrangements in *Arborea* to be distinct.

If the pod does indeed surround the units, this has potentially interesting implications for the production of micro-eddies and flow disturbance around the units (which have previously been hypothesized to explain community dynamics in Ediacaran fronds; Singer *et al.* 2012; Ghisalberti *et al.* 2014) potentially aiding nutrient uptake in these regions. Laflamme *et al.* (2018) noted similarities between *Arborea* morphology and feeding in extant pennatulaceans.

The anatomical arrangement we describe is distinct from both the fractal rangeomorphs (Narbonne 2004), which diagnostically require three orders of identical branching (Erwin *et al.* 2011), and also from the latest Ediacaran erniettomorph *Swartpuntia germsi*, which is characterized by a multi-vaned arrangement of featureless tubular branches (Narbonne *et al.* 1997). Recent studies suggesting a close phylogenetic relationship between the morphogroups Rangeomorpha, Arboreomorpha and Erniettomorpha (Dececchi *et al.* 2017; Hoyal-Cuthill & Han 2018) do not find support from our re-analysis of the anatomy of *Arborea*.

### *Growth*

The anatomical organization described above permits inference of the morphogenetic strategy of *Arborea*, which is informative when considering organismal affinities. The smallest, assumed to be youngest, specimens of *A. arborea* possess fewer branches than larger specimens. This suggests that branch growth and differentiation actively

occurred during the frondose stage of the organism's life cycle, with new tubular structures presumably developing and terminally differentiating as the frondose organism grew (rather than undergoing a single event of terminal differentiation). We find no upper size limit to *Arborea*, and thus suggest that it may reasonably be interpreted to have displayed indeterminate (size) growth, with no known maximum number of branches. Significant branch differentiation appears to have occurred in small specimens, with the smallest known specimens (~3.5 cm) possessing ~19 lateral branches. *Arborea* also shows a determinate (i.e. consistent and predictable) form within the studied population, with no evidence for aberrant branches (branches that are unusually long or short, or do not conform to the expected branching architecture; e.g. Kenchington *et al.* 2018). That the frond outline appears to change as specimen size increases, with the basal-most branches becoming relatively larger despite continued branch differentiation, suggests that new branches in *Arborea* differentiated from a (sub)apical generative zone (as indirectly inferred by Hoyal-Cuthill & Han 2018). We find no evidence for further, lateral generative zones.

An ordered fascicled branching arrangement requires a unidirectional guidance and pathfinding system along both the apico-basal and front–back axes. Pathfinding refers to the ability of a cell or group of cells to locate their final destination: neurons, for example, are able to find their destination by growing in permissive substrates and binding to adhesive cues (Raper & Mason 2010). Differentiation of the tubular structures (fascicles) into both branches and units occurs only after they emerge from the stalk wall, suggesting either the removal of an inhibitory signal within the stalk, or the presence of a positive differentiation signal in the stalk wall. The strategy outlined above is consistent with morphogenesis of branches in *Arborea* having occurred by localized outgrowth, as opposed to regional apoptosis (from an undifferentiated sheet). This is in line with many other forms of branching growth in extant eukaryotes, for example that seen in the alga *Ectocarpus* (Katsaros *et al.* 2006), or the bilaterian tracheal network (Affolter *et al.* 2009).

### *Phylogenetic placement of* Arborea

It is reasonable to assume that the anatomical complexity and large size of some *Arborea* specimens (~2 m in length) demonstrate that it was a multicellular organism, dwarfing even the largest multinucleate protists (xenophyophores). Indeterminate growth is compatible with several non-metazoan (e.g. Peterson *et al.* 2003) and metazoan (Sebens 1987) hypotheses of affinity, and is thus not considered an informative character here. *Arborea* lacks the serially quilted arrangement that has been considered diagnostic of the Vendobionta, and inferred in some rangeomorph taxa (Seilacher *et al.* 2003; Seilacher 2007). The constrained form of *Arborea* within populations exhibits no aberrant branches, a lateral margin bounding the branches, and determinate changes in form (i.e. a transition from a fusiform to a distally tapering frond outline). This is inconsistent with the growth pattern of many extant modular groups (e.g. plant or algal groups), and some multifoliate rangeomorphs, which are characterized by a lack of constrained form (Kenchington *et al.* 2018). The differentiation of new branches as *Arborea* grew is also incompatible with a fungal affinity, where a fruiting body undergoes one round of terminal differentiation (Umar & Van Griensven 1997). We therefore consider that to the exclusion of extant non-metazoan comparators, *A. arborea* was a total group metazoan.

The constrained form, presence of two main body axes, and extensive body regionalization is incompatible with a poriferan affinity, but such an axial arrangement is compatible with a eumetazoan affinity. We recognize differential preservation of anatomical features in *Arborea*, with structures in the interior of the organism being preserved, and external structures being entirely or partially missing in different specimens. This implies that these structures were distinct, and potentially composed of different original materials, and could indicate tissue differentiation: a eumetazoan character. Possession of a fluid-filled holdfast, potentially indicating a capacity for hydrostatic regulation, is also compatible with, but not unique to, a eumetazoan affinity. On the basis of all available evidence, we therefore propose that *A. arborea* lies within the Eumetazoa. Such a phylogenetic position has been presented previously (Buss & Seilacher 1994; Hoyal-Cuthill & Han 2018; though we disagree with the monophyletic clade of Ediacaran organisms favoured by these authors) but this reassessment of *Arborea* provides developmental and anatomical support. Our current knowledge of anatomical characters in *Arborea* is insufficient to permit further constraint of its phylogenetic position.

The fascicled internal anatomy of *Arborea* suggests that each lateral branch grew independently of its neighbours, implying developmental independence and thus conforming to the definition of biological modularity. Such an arrangement is comparable with extant taxa that possess colonial organization (e.g. various hydrozoans; Hyman 1940) and it is therefore entirely feasible that *Arborea* could represent an Ediacaran colonial eumetazoan (*contra* Landing *et al.* 2018). Coloniality has previously been predicted to be the plesiomorphic condition for the Cnidaria, with *A. arborea* itself (then termed *Charniodiscus*) proposed to lie at the base of the cnidarian tree (Dewel 2000; see also putative stem-group colonial cnidarians

from Cambrian Series 3; Park *et al.* 2011). However, more recent work (Zapata *et al.* 2015; Kayal *et al.* 2018) would suggest that this scenario is unlikely, with coloniality only being known in derived cnidarian positions. Ctenophores are not known to be colonial (we favour the view that Porifera represents the earliest diverging animal clade; Simion *et al.* 2017; Fueda *et al.* 2017) suggesting that the Ur-eumetazoan was a unitary organism. Coloniality is also noted as a derived condition within the Bilateria, with the only truly colonial phylum being the Bryozoa. If our interpretation of *Arborea* as a potentially colonial organism is correct, this may suggest that coloniality in eumetazoans was present in early-diverging groups. With no current evidence to tie *Arborea* to any crown group, this character could feasibly be present in early-branching positions of the eumetazoan stem-lineage, suggesting further (perhaps derived) excursions into the colonial state were possible, thus broadening the possible permutations of the eumetazoan ancestor.

## CONCLUSION

Reconstruction of the anatomy and developmental biology of *Arborea arborea* leads us to conclude that it represents a total-group eumetazoan. In addition to previously recognized morphological characters (Laflamme *et al.* 2018) we note a distinctive fascicled internal branching arrangement and a fluid-filled holdfast. The different taphonomic expressions of structures within the studied *Arborea* collection imply the possible presence of different tissue types, and thus tissue differentiation. We conclude that *Arborea* was a modular organism, and note that it displays characters consistent with (but not exclusive to) a colonial body-plan, something previously argued to have emerged in eumetazoans only in the Ordovician (Landing *et al.* 2018). Key differences between *Arborea* and rangeomorphs support morphological distinction between these frondose organisms, hinting at multiple independent excursions into frondose morphospace amongst early diverging animal groups.

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*Author contributions.* FSD and AGL designed and conducted the study. All authors were involved in drafting the manuscript and gave final approval for publication.

## DATA ARCHIVING STATEMENT

Data for this study are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.8t8h54h

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